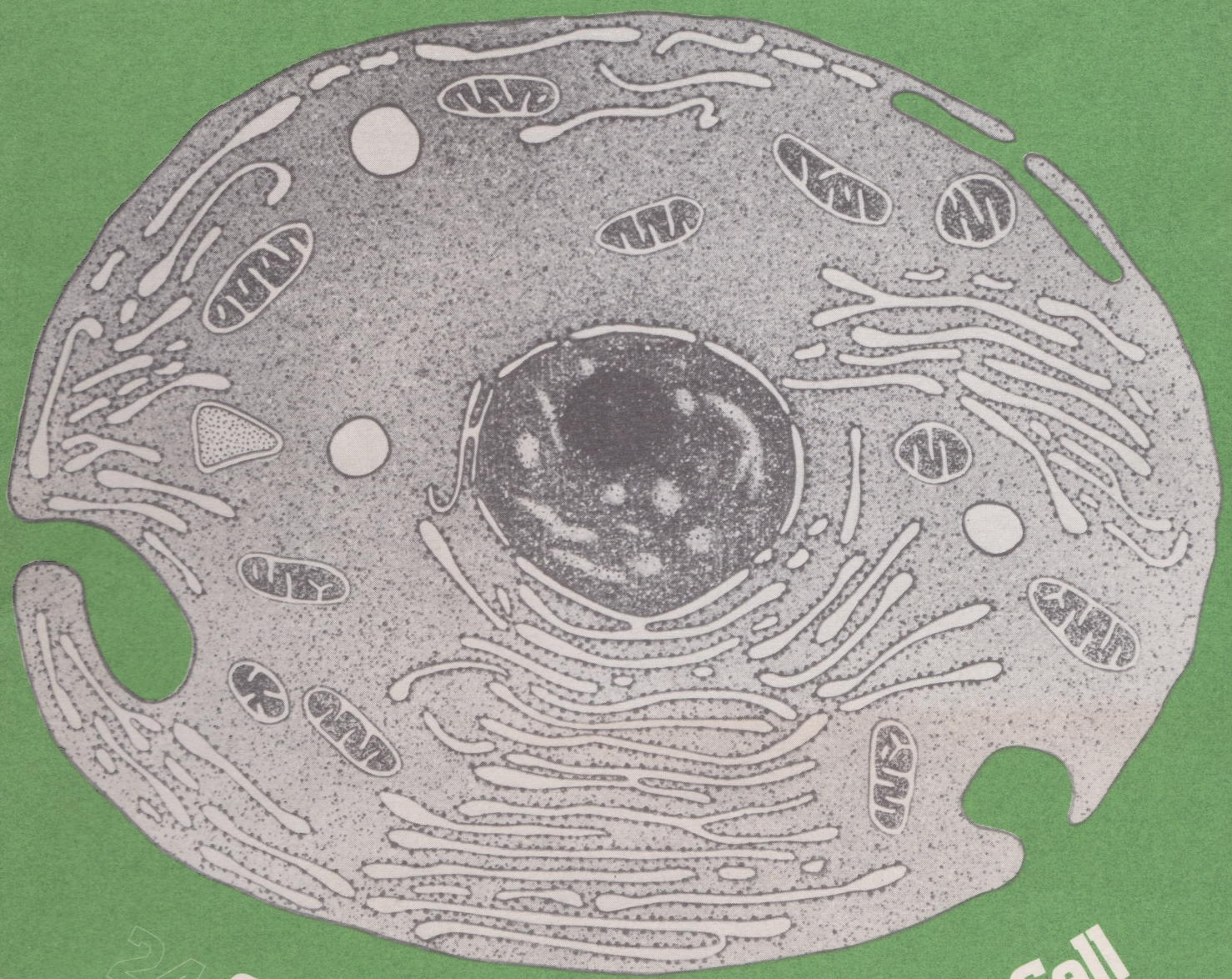
S101 Units 23 and 24

THE OPEN UNIVERSITY

Science : A Foundation Course

23 Cell Structure and the Chemical Components of Cells



24 Chemical Reactions in the Cell

The Open University

Science: A Foundation Course

Unit 23
Cell structure and the chemical components of cells

Prepared by the Science Foundation Course Team

The Open University Press

SCIENCE

S101 Course Team List

A note about the authorship of this text.

This text is one of a series that constitutes a component part of the Science Foundation Course. The other components are a series of television and radio programmes, audio-vision materials, home experiments, assignments and a summer school.

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The Science Foundation Course Team

Mike Pentz (*Chairman and General Editor*)

Francis Aprahamian (*Course Editor*)
David Broadhurst (*Physics*)
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Steve Swithenby (*Physics*)
Peggy Varley (*Biology*)
Kiki Warr (*Chemistry*)
Barrie Whatley (*BBC*)
Dave Williams (*Earth Sciences*)
Chris Wilson (*Earth Sciences*)

The following people assisted with particular parts or aspects of the Course:

Tel Bailey (*Tutor*)
Mary Bell (*Biology*)
Frances Berrigan (*IET*)
James Brennan (*Biology, Bridgewater State College, USA*)
Joan Brown (*Earth Sciences*)
Bob Cordell (*Biology*)
Beryl Crooks (*IET*)
Linda Fawke (*Tutor*)
Michael Gagan (*Chemistry*)
Ian Gass (*Earth Sciences*)
Ros Hamilton (*Tutor*)
Robin Harding (*Biology*)
Barbara Hodgson (*IET*)
Jen Horgan (*Tutor*)
Graham Jenkins (*Earth Sciences*)
Barrie Jones (*Physics*)
Paul Lyle (*Tutor*)
John Marshall (*Senior Counsellor*)
Bob Maybury (*Chemistry, UNESCO*)
Bob McConnell (*Earth Sciences, Virginia Polytechnic and the State University, USA*)
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Reg Melton (*IET*)
Yow Lam Oh (*Home Experiments, Caulfield Institute of Technology, Australia*)
Robin Russell (*Chemistry*)
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Peter Smith (*Earth Sciences*)
Russell Stannard (*Physics*)
Charles Turner (*Earth Sciences*)
Arthur Vialls (*BBC*)
John Walters (*Physics*)
Pete Wood (*Home Experiment Kit Coordinator*)
John Wright (*Earth Sciences*)
Martin Wright (*BBC*)

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* Introduced in the second Audio-vision sequence associated with this Unit, entitled 'Subcellular structure' (AC 92).

Study Guide

The previous Units in this Block have dealt with the biology of whole organisms. In Units 18–20 we discussed how they evolved to their present forms, and in Unit 21 how they interact with one another in communities. Only in Unit 22 did we begin to take organisms apart, in an attempt to see how they function. Here we discovered that all organisms are composed of *cells* and that in higher organisms these may be grouped together into *organs* like the heart and the leaf. In Units 23–25 we go into still more detail, and look at the components of cells themselves. The subcellular architecture which can be seen through the light or the electron microscope is described in Sections 1–5 of this Unit, and in the Audio-vision sequence you will study at the beginning of this Unit. The rest of this Unit, the whole of Unit 24 and much of Unit 25 is concerned with the molecular side of biology.

Sections 6 and 7 introduce the subject of biochemistry and some of the experimental approaches used in studying it. The remaining Sections describe the molecules found in the cell, and are essential background material for the whole of biochemistry. You will find it useful to have Units 14–17 and related Course material (that is, the last of the Chemistry 'boxes') to hand, when reading both Units 23 and 24.

The Audio-vision sequences for this Unit are in two parts. Before starting your study of the Main Text, you should work through the first part, which deals with plant and animal cells. For this you will need the Audio-visual cassette (AC 92), your viewer, the two filmstrips Nos. 23.1 and 23.2 and Figures A–D in the Notes for the Audio-vision sequence. The second part is on subcellular structure, and you should study this after reading Section 2. For this you will need cassette AC 92, the Notes and Photomicrographs 1–10, printed separately.

For the last part of Section 10 (10.5) you will need the stereoviewer and Filmstrip 23.3. A list of Filmstrip frames can be found on page 55.

The last part of Section 11 (11.7) is a two-hour Home Experiment, demonstrating the action of an enzyme (pepsin) on egg white. It is designed to illustrate key points in both Sections 10 and 11; writing up the experiment and answering questions related to it may form part of a TMA.

The television programme (TV 23) deals with the structure of animal and plant cells, and with some of the ways in which cells are studied.

1 The light microscope and the electron microscope

Study comment Before reading this Section you should have worked through the first part of the Audio-vision sequence, 'Cell structure and function'.

Filmstrips 23.1–23.2 and A–V Notes

light microscope

The light microscope consists essentially of a set of lenses through which ordinary light can be focussed on an object and made to give a magnified image of it. Its principal use is to make it possible to see more of the fine structure of the object being examined than it is possible to see with the naked eye. The magnification obtained may be useful, but unless the magnified image contains more detail than can be seen without using the microscope, then the instrument has only a limited value.

resolving power
resolution

The fineness of the detail that can be seen is termed the *resolving power* or *resolution* of the microscope. A microscope with a high resolving power will enable you to see two small objects close together as two distinct objects, whereas with a microscope of low resolving power, the image you will see will appear to be that of a single object. During the years in which light microscopes have been in use there have been considerable improvements, both in the magnification they can provide and in their resolving power. However, the resolution is both practically and theoretically limited. One of the main reasons for this is that resolving power is inversely proportional to the wavelength of the light used to form the image. Using the best quality microscopes and working with light in the visible

spectrum with a wavelength of 0.5 μm (0.5×10^{-6} m) it is possible to resolve two points 0.2 μm (0.2×10^{-6} m) apart. To improve resolution beyond this, 'light' of shorter wavelength still has to be used.

electron microscope

An instrument that does this is the electron microscope, in which the object is 'illuminated' with a beam of electrons. You have learnt that electrons are tiny, negatively charged particles and that they are one of the constituents of atoms. Basically the idea behind the electron microscope is that a beam of electrons is directed at the specimen and 'illuminates' it much as ordinary light does in the light microscope. If you find this surprising it may help you to realize that there is an analogy here between electrons and photons. As you saw in Unit 9, light propagates like waves and interacts with matter like particles. The same can be said of electrons, and as you will see in Unit 29, this wave–particle dualism has great theoretical significance. It also has practical significance for cell biologists, because the wavelength of an electron beam depends on the momentum of the electrons and can be made extremely short. For instance, an electron wavelength of 5×10^{-12} m can be produced, which is about 10^5 times smaller than the wavelength of visible light.

The electron microscope utilizes these properties. The electron beam is focussed by electrostatic and magnetic lenses and produces an image of an object put in its path. The resolving power of an electron microscope is thus thousands of times greater than that of a light microscope, and a modern electron microscope can easily resolve points down to about 10^{-10} m apart. The image produced of the object being studied is enlarged, just as the image produced in a light microscope is, but in the case of the electron microscope magnifications of up to × 500 000 are possible, compared with magnifications of up to × 1 500 with light microscopes.

2 Some techniques used in the microscopic examination of cells

Before cells can be examined microscopically they generally have to be specially prepared. The most widely used techniques are those in which the cells are prepared for examination by transmitted light or by a beam of electrons that passes through them. Almost always cells are thicker than is desirable for good image formation so they have to be cut into thinner slices or *sections*. However, before this can be done a variety of other procedures have to be carried out, whether the specimens are to be examined by light or electron microscopy.

First the cells have to be killed and *fixed*. You might wonder why fixation is necessary. Imagine some of the problems you might have to overcome if you attempted to find out what was inside a raw hen's egg, and at the same time tried to find out how the contents were arranged. Simply opening the egg in order to inspect the contents would result in a mess. However if the egg was boiled first and then opened, if in fact the contents of the egg were fixed, inspection would become much easier because the fluid contents would have been solidified, we hope retaining the structure of the raw egg. There are a wide variety of fixation processes in use but they all have the same purpose, namely, to make it easier or possible to study the cell while preserving its structure from change and decay.

After cutting the cells into sections it may be necessary to stain the sections. The object here is to assist microscopic examination by taking advantage of the chemical differences between subcellular organelles. In light microscopy, by using dyes that react with some structures but not with others, or by using chemicals that react with some chemicals present in the fixed cells to form coloured compounds, but not with others, particular parts of the cell are picked out. In electron microscopy solutions of metal salts are used in place of dyes. Because these salts bind differently to different components inside cells, after treatment some components will transmit electrons more than others. These differences produce lighter or darker parts in the photographic image produced by the instrument.

For material that is to be examined with an electron microscope, an important additional step is necessary: the fixed and stained section has to be dried, because electron microscopy requires the specimen to be examined in a vacuum. Material that is to be examined with the light microscope is often dehydrated, the water in the specimen being replaced by other chemicals such as glycerine or a resin.

The image formed by either the light or electron microscope can be photographed. The photographs of cells you have examined so far and those you will examine later are photographs of such images. But, as you have already seen in the first Audio-vision sequence, the art of the microscopist does not stop with the production of a highly magnified finely detailed image, it also includes the skills of interpretation. We will return to this topic after you have studied the next section of the tape which deals with the detailed structure of cells—with their ultrastructure.

Before continuing with your study of this Unit you should work through the Audio-vision sequence 'Subcellular structure'. For this you will need the cassette AC 92, and Plates 1–10 and Figures 1–4 which are printed separately.

A–V Notes and Photomicrographs 1–10

3 The size of subcellular organelles

So far, nothing has been said about the size of structures found in cells. Most cells are too small to be seen with the naked eye. However, with the light microscope, cells and their nuclei can easily be observed. The structure of the cells' membranes, or of their mitochondria however cannot be seen. As you have noticed for yourself, greater detail can be seen using the electron microscope. If you measure the diameter of the image of the mitochondrion marked X in Plate 2 of the Audio-vision Notes you will find that it is between 13 and 19×10^{-3} m (i.e. between 13 and 19 mm).

To find the real diameter, the measured length must be divided by the magnification of the photograph, in this case $\times 40\,000$. So the diameter of this mitochondria is about:

$$\frac{16 \times 10^{-3}\,\text{m}}{40\,000} = 4 \times 10^{-7}\,\text{m} = 0.4\,\mu\text{m}$$

You may find that in some textbooks the lengths of cell structures are given in Ångstrom units (abbreviated to Å). The relationship between Ångstrom units and the SI unit of length (the metre) is 1 Ångstrom unit (Å) $= 10^{-10}$ m.

4 Artefacts

From your study of cells and their ultrastructure, two questions at least should have been raised in your mind: what do all these things do in cells? do these structures really exist?

The first question is answered in several places in this Course. The functions of mitochondria and chloroplasts are discussed in Unit 24, and you will learn about those of ribosomes and of the nucleus in Unit 25. The functions of the plasma membrane, endoplasmic reticulum and tonoplast are not discussed in this Course, although they are included in the summary of cell structure in this Unit, Section 5.

The second question, whether the structures seen inside cells really exist, is very difficult to answer. The question is really in two parts. Do the structures really exist in killed and fixed cells? If so, do the same structures exist in living cells?

Structures such as the plasma membrane, the chloroplasts and the nucleus certainly do exist. In whatever way cells are prepared for examination, however they are killed, microscopic examination always reveals the presence of these structures. In some cases and by using special techniques, living cells can be examined microscopically and again these structures can be seen. It is with the smaller subcellular organelles that the difficulties really arise. As you have seen, mitochondria are just about visible when cells are examined with the light microscope but neither ribosomes nor the endoplasmic reticulum are: these can only be seen in electron-microscope studies. So it is possible that these subcellular organelles are *artefacts*—objects produced in the cells as the result of the chemical and/or physical treatments used in preparing them for examination. At present it is not

artefacts

possible to examine living cells with the electron microscope, so a direct check on the existence of these smaller subcellular organelles cannot be made. However, it is possible to use a range of different treatments to kill, fix cells and stain sections of the cells. When the resulting photographs of these different sections are compared there are often differences in the appearance of the subcellular organelles. Ribosomes and endoplasmic reticulum are usually visible irrespective of the pre-treatment used, so on these grounds it is believed that they really do exist. There is also general agreement about the structure inside mitochondria and chloroplasts.

Whether the structures that are agreed to be present in sections of killed, fixed and stained cells correspond to any structures in living, functioning cells is another matter altogether. As you will see in the TV programme associated with this Unit (TV 23), chloroplasts and even the nucleus move about in living cells. If the endoplasmic reticulum really is a complicated set of interconnected membranous sheets, then rapid, long-distance movements of the chloroplasts is not compatible with what we imagine the endoplasmic reticulum to be. Similarly, if the nuclear membrane is attached to the endoplasmic reticulum, as it certainly appears to be from electron-microscopic studies, then about the last thing we would expect the nucleus to be able to do is to spin round, and yet spinning movements have been observed! In both these cases, different lines of evidence from which conclusions about the detailed structure of cells can be drawn appear to lead to different conclusions.

While this creates an uncomfortable situation, the same sort of situation has occurred in other areas in biology and in other scientific disciplines. Just as the steady accumulation of additional evidence eventually allowed astronomers to choose between a geocentric and a heliocentric model for the Solar System, so no doubt it will one day be possible to decide which of the details of subcellular structure correspond to the real structure of living cells and which are artefacts.

While the problem of artefacts can be well illustrated in the field of microscopy, it is not confined to it. As you will see from later Sections of this Unit, biological molecules may easily alter their properties once they have been removed from the carefully defined environment of the cell. In biochemical experiments too therefore, you should always be aware of the dangers inherent in extrapolating results from work done *in vitro*, outside the cell (e.g. in test-tubes), to situations inside the living cell (*in vivo*).

in vitro
in vivo

5 Summary of cell structure

1 Cells are the units of which the bodies of living organisms are made. The number of cells present ranges from one up to several millions.

2 Every living cell is surrounded by a plasma membrane inside which is a viscous fluid, the cytoplasm.

3 On the basis of their detailed structure cells are classified as either prokaryotic or as eukaryotic. The principal differences between them are as follows.

prokaryotic cells
eukaryotic cells

Prokaryotic cells	Eukaryotic cells
(a) No recognizable nucleus separated from the cytoplasm by a nuclear membrane	A recognizable nucleus is present and is surrounded by a nuclear membrane
(b) Mitochondria are absent	Mitochondria are present
(c) Chloroplasts are absent	Chloroplasts may be present

4 Prokaryotic cells are simpler in their structure than eukaryotic cells and are the basic units of the simplest living organisms, the bacteria.

5 Eukaryotic cells are the basic units of the bodies of animals and plants, including the single-celled types and the fungi.

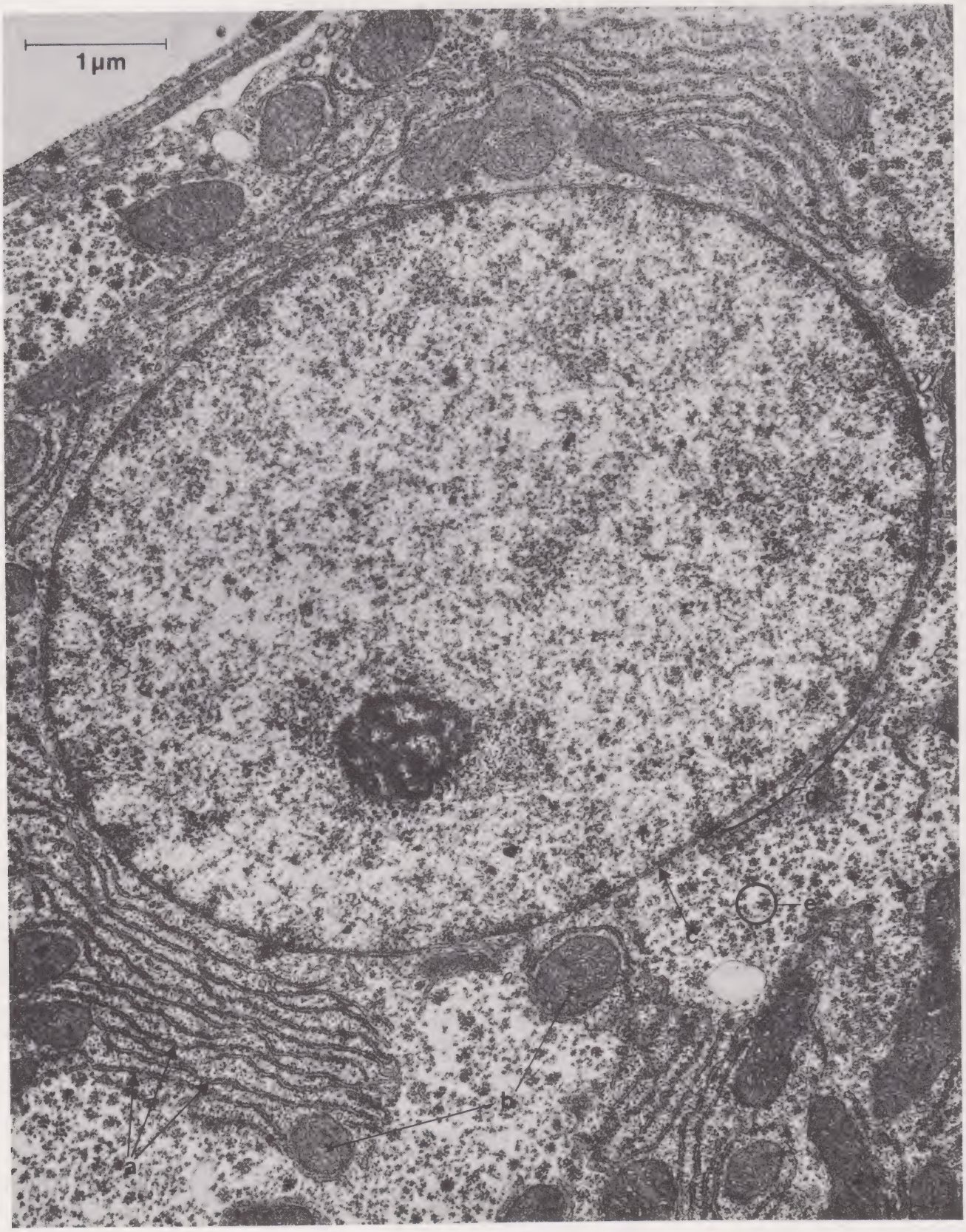
6 Eukaryotic cells themselves can be classified into two major types. Those of animals generally do not have a large fluid-filled vacuole inside and do not have

cell walls. Those of plants usually do have a cell wall around the cell and when mature usually do have a large fluid-filled vacuole inside.

7 On the basis of their fine or ultrastructure, animal cells and plant cells are very similar. Both types of cell are surrounded by a cell membrane. They contain one nucleus (seldom more), ribosomes, organelles which include mitochondria and endoplasmic reticulum, and may, in the case of plant cells, include chloroplasts.

8 The shape and detailed structure of both plant and animal cells is very varied because individual cell structure is related to the principal function the cell performs.

FIGURE 1a For use with SAQ 1.



5.1 Objectives of Sections 1–5

Now that you have studied Sections 1–5, you should be able to:

(a) Identify endoplasmic reticulum, mitochondria, chloroplasts, the cell membrane and the nuclear membrane in photographs.

(b) Estimate the size of cellular structure in photographs, given a scale or magnification.

To test your understanding of these Objectives, try SAQs 1 and 2.

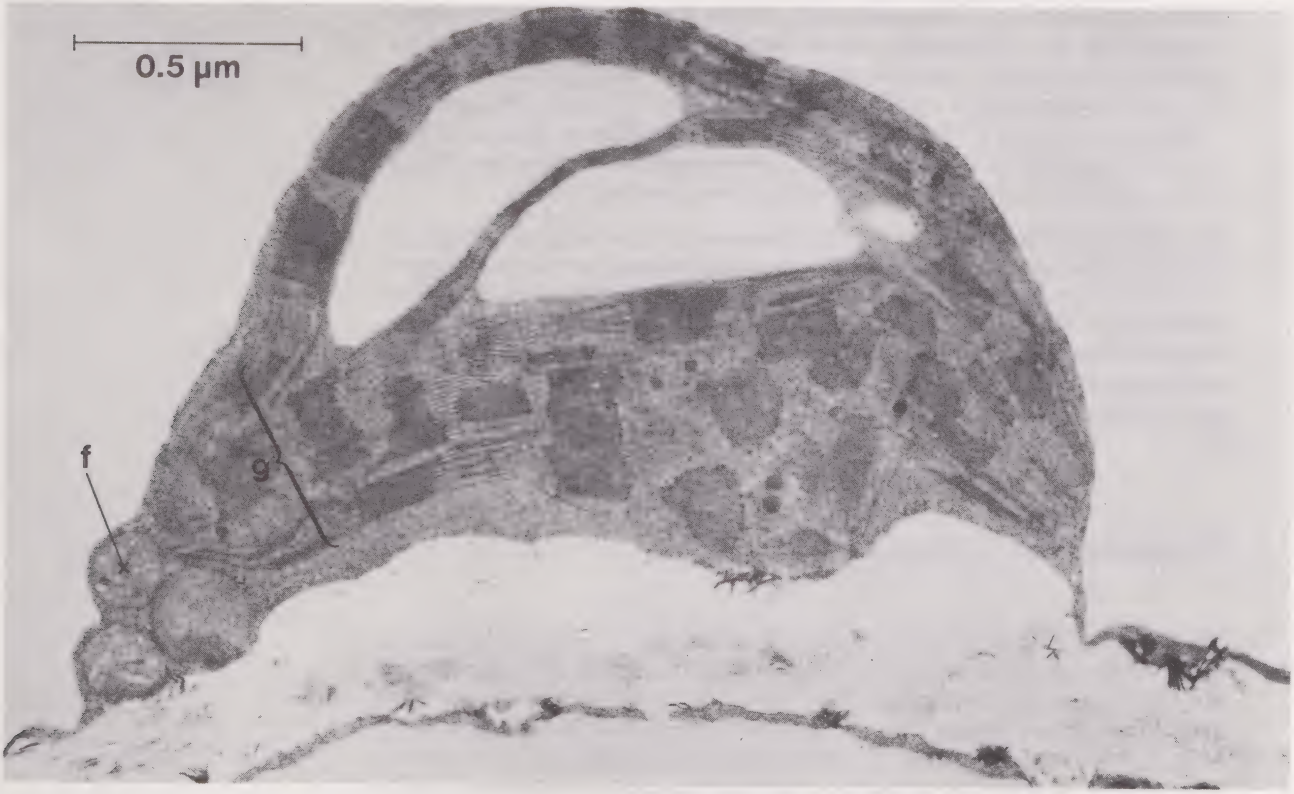
SAQ 1 (*Objective* (*a*)) (a) Identify each of the items a–g in Figures 1a and 1b.

(b) Is the cell, part of which is shown in Figure 1a, a eukaryotic cell? Give reasons for your answer.

(c) Is the cell, part of which is shown in Figure 1b, an animal or a plant cell? Give reasons for your answer.

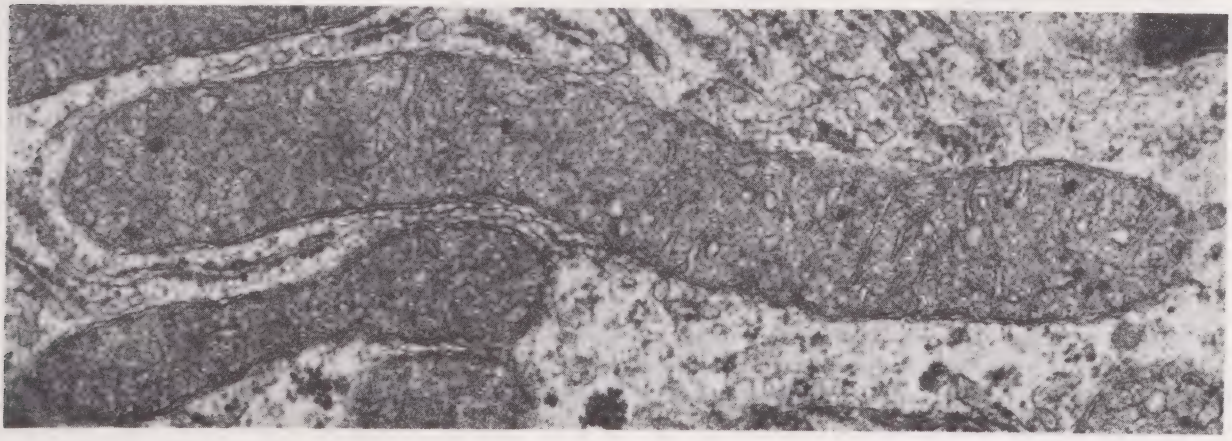
SAQ answers begin on p. 55.

FIGURE 1b For use with SAQ 1.



SAQ 2 (*Objective* (*b*)) Identify the organelle in Figure 2. The image of the organelle is 30 000 times life size. What is the real width of the organelle?

FIGURE 2 For use with SAQ 2.



6 Introducing biochemistry

Study comment Both this Section and Section 7 may be read through fairly rapidly. They are intended to indicate what the subject of biochemistry is about, and how it is approached experimentally.

It is the interaction of molecules in the cell that lies at the root of the various activities that distinguish living material from non-living. The purpose of biochemistry is to relate these activities first to the kind of molecules found in living organisms, then to the kind of chemical transformations these may undergo, and finally to the way these transformations may be controlled.

The roots of biochemistry were not established until the nineteenth century, when scientists finally became convinced that there is nothing magical about the chemistry of life. The molecules of living organisms obey the same laws of physics and chemistry as those of non-living material. They can be extracted from the cell, put into test-tubes, and even made to perform there many of the reactions normally carried out in the cell. There are however particular difficulties in working with biological molecules. One is that they tend to be very large and have complex structures that readily fall apart outside the precisely defined environment of the cell. Biochemists cannot therefore use many of the standard techniques of chemistry, especially where these involve heating to high temperatures and subjecting cell components to other extreme conditions. Another difficulty is that most of the interesting biological molecules are present in only very small amounts in the cell. After much labour, just a few micrograms of pure compound may be obtained from several hundred grams of tissue.

Because of the delicacy and rarity of their materials, biochemists are very dependent on techniques. The great achievements of biochemistry over the past 30 years have relied very much on the development of specialized equipment, capable of isolating and observing biological molecules under conditions as near as possible to those of the living cell. This is why modern biochemistry laboratories house so many large and expensive 'black boxes'. One such universal piece of equipment is the centrifuge, to be described in the next Section.

7 Preparing cell extracts

The fine detail of cellular structure and occasionally even individual molecules can be seen with the light microscope or the electron microscope, as you saw in the Audio-vision sequence and the Audio-vision Notes. However, only a certain amount of cell chemistry can be learned by just looking down a microscope. The bulk of our information comes from collecting and analysing the contents of a large number of cells. Cell membranes are ruptured, giving a suspension of cell contents known as a *homogenate*. If a fairly ferocious instrument, like a kitchen liquidizer, is used for this purpose, the homogenate will be a mixture of the contents of all subcellular organelles. Such preparations form the starting point for many biochemical purifications and are often adequate for simple investigations into cell chemistry.

homogenate

However, it is possible to disrupt the cell membrane by gentler techniques, which leave intact the membranes of subcellular organelles. In this case, we may separate out the mitochondria, nuclei, and so on, and study their individual chemistry. One way of separating the various organelles after gentle homogenization is to treat the cell homogenate as if it were a suspension of very fine sand in water. If such a suspension were shaken up and then allowed to settle, the largest grains would fall to the bottom first, then the medium ones, and some very fine grains would remain suspended in water even after several hours. The rate at which the particles fall depends on several factors: their size, their density compared with the surrounding solution (a particle of cork, however large, would not fall to the bottom as its density is less than that of water) and finally, the gravitational acceleration to which they are subjected. In our imaginary experiment with sand and water, the gravitational acceleration is constant so long as the container holding the suspension is standing still; therefore the particles may take several

hours to sediment. But ways exist of increasing the velocity with which they move towards the bottom of the container. Accelerating the container upwards in a lift or a rocket would be one way. Another would be to whirl the container round very fast at the end of a long arm (see centre of Figure 3). This is the basis of *centrifugation*, a technique used when training fledgling spacemen to simulate the increased force of gravity during rocket acceleration; it is also used in domestic spin dryers.

centrifugation

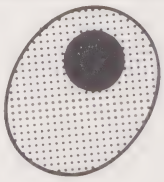
In the biochemistry laboratory, modern centrifuges are able to spin homogenates at speeds of 60 000–70 000 rpm (revolutions per minute). This is the equivalent of applying a force of up to 500 000 times gravity to the cell homogenate. The particles in suspension are now forced to settle under the action of a greatly increased gravitational force, and therefore reach the bottom of the container (the centrifuge tube) in a reasonable time. Returning to our original analogy, the subcellular organelles of the homogenate behave like different-sized grains of sand, and can thus be separated.

In the technique known as *differential centrifugation* the homogenate is suspended in a solution whose density is not very different from that of water. The suspension is then centrifuged for particular combinations of speed and time. At the lowest speed and shortest time, the largest organelles (cell nuclei) collect at the bottom of the tube and can be withdrawn. As speeds and times are increased, so smaller and smaller organelles can be collected (see Table 1). The molecules in the different subcellular fractions can then be chemically analysed.

differential centrifugation

separation of organelles

TABLE 1 Separation of subcellular organelles by differential centrifugation. The 'Time' column relates to the *duration* of the centrifugation. The column headed 'Ratio between centrifugal acceleration and gravity' relates to the *speed* of centrifugation. The organelles are described in the text.

Organelle	Shape	Time/min	Ratio between centrifugal acceleration and gravity
nuclei		10	800
mitochondria		15	12 000
lysosomes		15	25 000
ribosomes		60	300 000

Sometimes quite different organelles have rather similar sizes, and are therefore difficult to separate by differential centrifugation. It was to purify such organelles that the technique of *density-gradient centrifugation* was developed. Here the homogenate is not introduced straight into the centrifuge tube, but poured carefully on top of a series of solutions already in the tube. Each solution has a

density-gradient centrifugation

different density (see top of Figure 3). The solutions contain sucrose (table sugar); the densest solution, which sits at the bottom of the tube, is that with the most sucrose. When the centrifuge tube is now spun, the subcellular particles in the homogenate will come to rest in the layer of sucrose solution whose density corresponds to their own. Particles of similar size but different density can therefore be separated, as shown at the bottom of Figure 3.

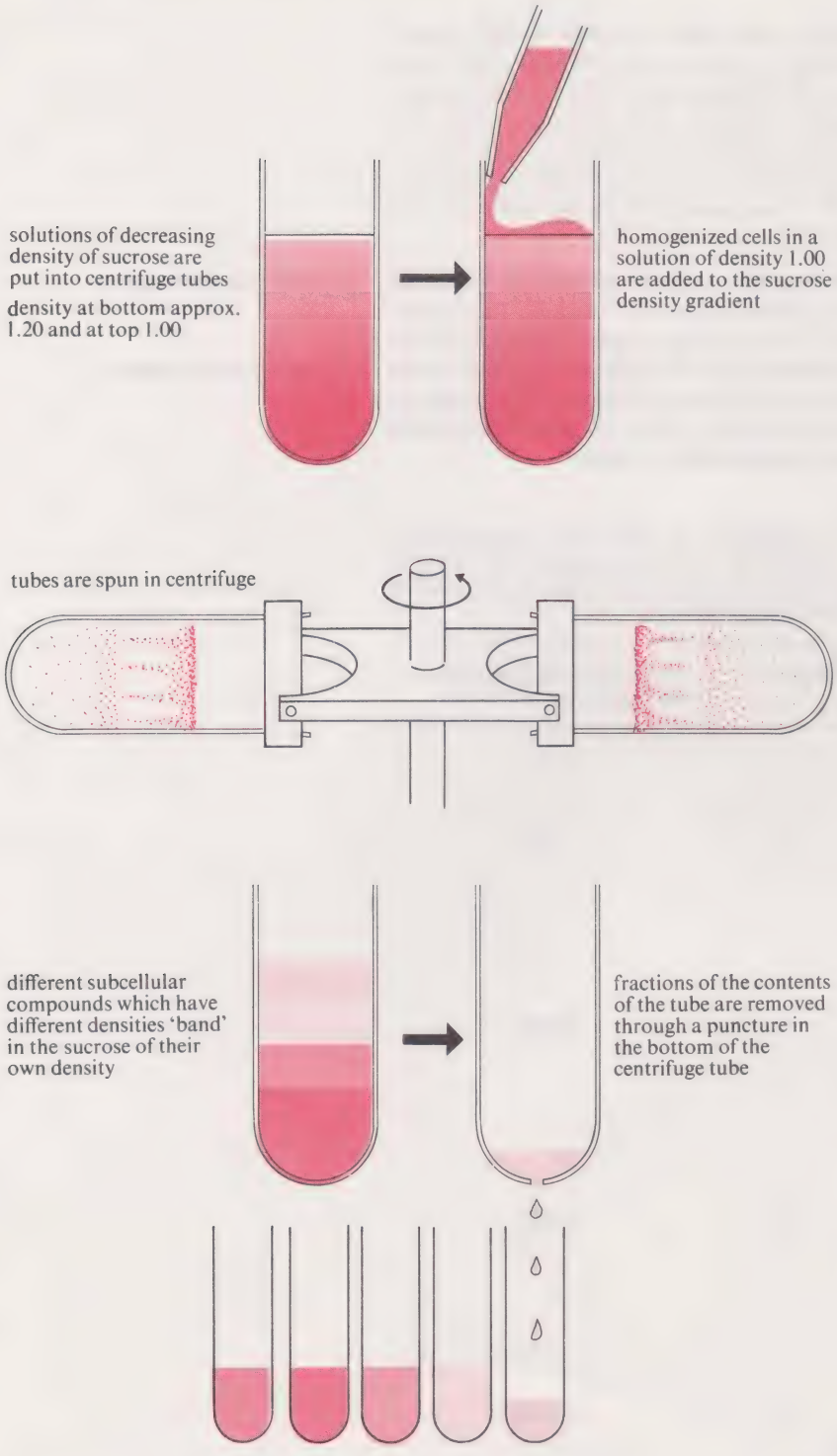


FIGURE 3 Outline of the technique for the separation of subcellular organelles by density-gradient centrifugation.

Without a proper understanding of the physical principles behind centrifugation, no biochemist can take full advantage of its powers of separation. Armed with such insight, de Duve and his colleagues in 1953 were able to demonstrate the heterogeneity of the so-called mitochondrial particles collected by differential centrifugation. In reality, this part of the homogenate contains two quite different particles of very similar size—mitochondria and lysosomes. The mitochondria, as we shall see in Unit 24, are concerned almost exclusively with energy production. The lysosomes, however, carry a battery of biological catalysts capable of destroying all major cell components. Normally safely contained within a fragile

membrane, these 'suicide bags' come into action for quite different purposes. For example, when the cell dies, the lysosomes burst and the destructive contents released from them break down the cell contents into small molecules which are then available for building the new contents of new cells. In other circumstances, lysosomes may engulf and digest foreign particles (such as bacteria) which are taken inside the cells.

Fortunately, lysosomes and mitochondria have different densities, despite their similarity in size. They can therefore be separated by density-gradient centrifugation, but only by judicious manipulation of all possible variables in the equation that governs sedimentation. This equation is given in Appendix 1, which you could now read if you are interested in seeing how closely biology and physics are interlinked.

Centrifugation is just one step in the process of separating out and analysing the molecular components of the cell. We shall turn to the results of some of these analyses in Section 8.

7.1 Objective of Section 7

Now that you have studied this Section, you should be able to:

(a) Choose an appropriate method of centrifugation, given the relative sizes and densities of organelles in a homogenate.

To test your understanding of this Objective, try SAQ 3.

> **SAQ 3** (*Objective* (*a*)) Homogenate A contains three organelles, *a*, *b*, *c*. Organelle *a* is about ten times the size of *b*, and about a hundred times the size of *c*. The three organelles have very nearly the same density.
>
> Homogenate B contains three organelles, *x*, *y*, *z*, which have very nearly the same size, but have densities of 1 050, 1 170 and 1 280 $kg\,m^{-3}$ respectively.
>
> What method of centrifugation would you use to separate the organelles in each of these homogenates?

8 Classes of molecules found in the cell

> **Study comment** This short Section introduces all classes of compound found in the cell. Table 4 summarizes these, in a preview of the biochemistry to be covered in Units 23–25. You should *not* expect to understand this Table fully now, but should aim to have mastered most of the information in it by the end of Unit 25. You may also find it useful to refer back to as you read through Units 23–25.

In this Section we move yet further down the scale, from organelles to molecules. Our first task is to analyse chemically the substance of living organisms, and to ask is there anything unique, chemically, about living organisms. To determine the proportions of the various chemical elements present in, say, the human body is comparatively simple. It uses the same techniques that analytical chemists apply to the simpler problems presented by minerals. Table 2 contains the summary of such an analysis for an 'average' human.

It is immediately apparent from this Table that three elements—oxygen, carbon and hydrogen—account for 93 per cent of the total body weight. The analysis of just how the elements are combined into molecules is a more complex task, and one that is in no sense complete even today. It is possible, however, to describe the *classes* of compound present with some precision. The crudest, and primary division may be illustrated by an experiment in which a cell homogenate is simply filtered to separate the soluble from the insoluble material. The soluble fraction now contains nearly all the small molecules (those, that is to say, with a relative molecular mass of anything up to 10 000 or so), whereas the insoluble residue, typically whitish or pale brown in colour and rather pasty in consistency, contains the large molecules (with relative molecular masses up to several millions). These

TABLE 2 Elemental composition of the human body by weight.

Element	Percentage of body weight
oxygen	65
carbon	18
hydrogen	10
nitrogen	3
calcium	2
phosphorus	1.1
potassium	0.35
sulphur	0.25
sodium	0.15
chlorine	0.15
magnesium, iron, manganese, copper, iodine, cobalt, zinc	traces

macromolecules are also called biopolymers, for reasons that will shortly become clear. They include the *proteins*, *nucleic acids* and *polysaccharides*. Also present in the residue are molecules known as *lipids*—all of which are insoluble in water but will dissolve in nonpolar compounds such as chloroform. In the next Section we shall be describing the small organic molecules which are the components from which these four main classes of molecule—protein, nucleic acid, polysaccharide and lipid—are made. Table 3 indicates their relative abundance in the human body.

macromolecule

lipid

For most biologists it is the giant macromolecules which are the really interesting and exciting ones, but here we must begin by looking briefly at the commonest and most important of the simple molecules present in the living cell. Apart from large quantities of water, and the simple inorganic ions (such as positively charged potassium, sodium, calcium and magnesium, and negatively charged chloride and phosphate), the small molecules of the cells are all organic compounds. Many of these are the building blocks from which the larger macromolecules are put together. The most important of the small organic molecules are the sugars, the fatty acids, the amino acids and the purine and pyrimidine bases. The relative abundance of small organic molecules and inorganic compounds is shown in Table 3.

TABLE 3 Classes of compound found in the human body.

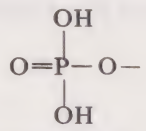
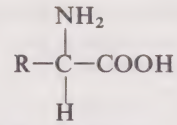
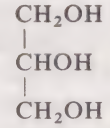
Substance*	Percentage of body weight
water	60–80
protein	15–20
lipid	3–10
polysaccharide	1–15
small organic molecules	0–1
inorganic compounds other than H_2O	1

* Nucleic acids do not feature in this Table, being quantitatively only a minor component of the human body.

All these classes of small molecules in the cell are listed in Table 4, which summarizes some of the essential points given in this Unit and in Units 24 and 25. We do not intend that you should remember all the names or formulae of the molecules described here. You should be able however to remember in general terms, which *classes* of substance are present within the cell, some of their general properties, and one or two examples of each (by name, not formula, unless it helps you to memorize this).

You should also be clear which small molecules are the building blocks for protein, polysaccharide, fat and nucleic acid. We shall now describe the structure of these component small organic molecules in more detail.

TABLE 4 Small molecules found in the cell.

General class	Group name	Typical examples	General formula	Examples of special roles in the cell
Inorganic ions	cations	sodium potassium calcium magnesium	Na^+ K^+ Ca^{2+} Mg^{2+}	inhibitors and activators of enzymes (Unit 23)
	anions	phosphate	 represented Ⓟ for short	phosphate groups participate in many biochemical reactions (Unit 24)
Organic acids	carboxylic acids	citric acid pyruvic acid etc. fatty acids	RCOOH	intermediates in breakdown of sugars and in biosynthetic reactions (Unit 24); long-chain fatty acids are present in *lipids* (Unit 23)
	amino acids	20 different ones, e.g. glycine alanine (see Table 5)	 where R may be one of about 20 (and differs from the R in carboxylic acids)	individual building blocks of which *proteins* are composed (Unit 23)
Organic alcohols 1 Sugars	6-carbon (6C) sugars	glucose fructose	$C_6H_{12}O_6$; carbon atoms present as chain or ring	building blocks of which *polysaccharides* are composed (Unit 23); oxidation of glucose is cell's main source of energy (Unit 24);
	5-carbon (5C) sugars	ribose deoxyribose	$C_5H_{10}O_5$, $C_5H_{10}O_4$; carbon atoms present as chain or ring	components of *nucleic acids* (Unit 25); ribose also features in photosynthesis (Unit 24)
2 Glycerol	3-carbon (3C) compound			component of all *fats* (Unit 23)
Purine and pyrimidine bases		adenine guanine cytosine thymine, uracil	see Figure 6	adenine combines with ribose and phosphate to give ATP (Unit 23); all five bases combine with 5C sugars and phosphate to form building blocks of which *nucleic acids* are composed (Units 23 and 25)

9 Small organic molecules in the cell

Study comment This Section contains vital background information for the rest of the biochemistry in this Course. It also builds on Units 16 and 17, which you should have to hand.

Even if this Section appears to you as a rather dry catalogue, do not be disheartened. It will stand you in good stead for the more exciting bits of biochemistry to follow. The detail with which you are expected to remember the information is pinpointed in the Objectives at the end of each subsection, which you should read carefully, and also in the study comments at the beginning of each subsection. Note also that additional Objectives and SAQs relating to Section 9 as a whole appear at the end of the Section.

9.1 Sugars

Study comment Since sugars are found predominantly as rings, you must be able to understand the convention used to depict these. This is shown in Figure 4, which is a key figure for this subsection. Apart from this, the most important aspect of sugar chemistry for our purposes is the reaction of sugars with phosphate. You should also be clear about the system of numbering carbon atoms—especially the meaning of C-1, C-4 and C-6.

monosaccharide
polysaccharide
carbohydrate

Simple sugars are usually known as *monosaccharides*, to distinguish them from the *polysaccharides* formed by their polymerization. Both mono- and polysaccharides may also be called *carbohydrates*. They contain just three elements, C, H and O, and can be synthesized by plants from carbon dioxide and water. As you can see from Table 4, they can be classified as alcohols (see Units 16 and 17) because of their many OH groups. The general formula of a sugar is $C_nH_{2n}O_n$. In the commonest ones $n = 5$ or 6 (giving $C_5H_{10}O_5$ or $C_6H_{12}O_6$). The most abundant and probably best known to you of the simple sugars is glucose—$C_6H_{12}O_6$.

The reactive groups of glucose are most clearly seen in the two open-chain formulae shown in Figures 4a and 4b. The six carbons are arranged as a short chain. By convention, carbon 1 (C-1) is either the one carrying the carbonyl group (C=O) —as happens in glucose—or the carbon at the end of the chain that lies nearest to this group. Note that in Figure 4b the structure shown in Figure 4a has been simplified. The C symbols for carbons 2–5 have been omitted, as have the single H atoms attached to them. The OH groups on carbons 2–5 are shown simply as straight lines*. As you will see, it is the orientation of these OH groups (the straight lines in Figure 4b) that distinguishes one 6-carbon sugar from another. In glucose, all the carbons except C-1 carry an OH group and one (or two) H atoms. It is the group of which C-1 forms a part, the carbonyl ($>$C=O), that is the most reactive part of the chain. In fact it is so active that it interacts with atoms further down the same chain, forming one of the two 6-membered ring structures shown in Figures 4d and 4e. In solution therefore, the open-chain structure is present in only *very small* quantities.

To visualize how the ring closes, we may *imagine* (though this is far from representing the true mechanism of the reaction) that the H atom of the C-5 OH group jumps to the O already on C-1 (thereby forming a different OH group, which is shown shaded grey in Figure 4). The O left on C-5 becomes the ring oxygen (outlined in red in Figures 4d and 4e) and forms a link between C-1 and C-5. Of the five carbons in the glucose ring, four have OH groups attached to them (C-1, C-2, C-3 and C-4), while the fifth (C-5) carries the CH_2OH group that includes C-6.

glucose

Glucose is but one of many possible monosaccharides with *substituent groups* (such groups include the CH_2OH or OH that substitute for H) attached to these

* This is *not* the convention used in the chemistry Units where a single bond by itself represents —H. You will come across both these conventions in biochemistry textbooks.

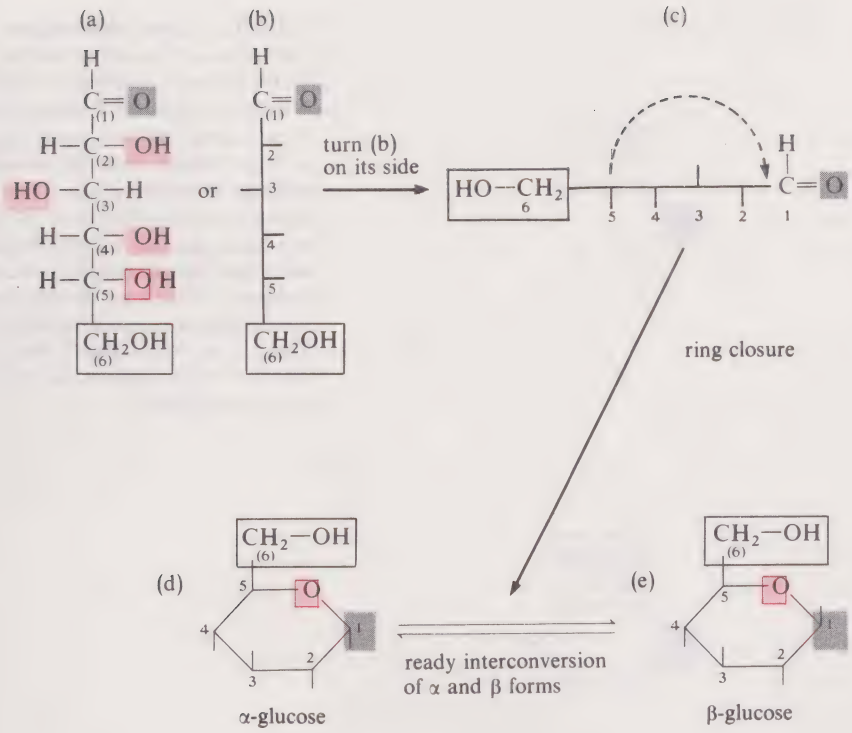


FIGURE 4 Relationship between straight-chain and ring structures of glucose. You are not expected to remember every detail of this Figure; the important bits have been picked out by shading or boxes. Pink shading emphasizes the position of OH groups on the straight-chain formula (a). (b) is a shorthand version of (a), showing the OH groups on carbon atoms 2–5 simply as straight lines (see the text for an explanation of this convention). In the ring structures (d) and (e) the OH groups are represented by vertical lines. The reactive C=O group in (a), which becomes the C-1 OH in (d) and (e), is emphasized by grey shading. Note the difference between the two ring structures α and β. To help you to follow the transition from straight chain to ring, we have outlined C-6 throughout by a black box and have outlined in red the O on C-5 that becomes the ring O.

particular ring carbons*. The source of variety is the *orientation* of substituent groups relative to one another. (This orientation derives from the angle between carbon bonds in real space, and can be related to the straight-chain formula by simple rules.) The two 6-carbon sugars, galactose and glucose, shown together in Figure 5, are a good pair to illustrate this point. They differ solely in the arrangement of groups about C-4. Yet in the cell they behave as quite different molecules, and they are indeed different sugars. Although their structures look so similar, they cannot readily be interconverted. Two covalent bonds would need to be broken and remade, and this, as we shall see in the next Unit, cannot be done without the intervention of enzymic catalysis.

The only sugars that can be interconverted without catalysis are those that differ in their configuration at C-1. These are the alpha (α) and beta (β) forms, which are shown for glucose in Figures 4d and 4e. The sugar ring can open fairly easily at C-1 and then re-close, giving the alternative ring structure. By convention, α-sugars are drawn with the C-1 OH shown pointing *downwards*, and β-sugars are drawn with it pointing *upwards*.

The sugars you will come across in this Course are mainly derived from one of three simple monosaccharides—glucose, fructose or ribose. Of these, glucose is the only 6-carbon sugar with its carbonyl group on C-1. You can see this by comparing these three sugars as shown in Figure 5. *Fructose*, like glucose, is a 6-carbon sugar, but differs in the position of its carbonyl group. As you can see from its straight-chain formula, the fructose carbonyl lies on C-2, not C-1. Ring closure in fructose, as in glucose, involves reaction between the carbonyl group and an OH group lower down the sugar chain. Since there is one less carbon between C-2 and C-5 of fructose than between C-1 and C-5 of glucose, the fructose ring is one atom smaller, that is, the ring has five members, not six. (Both α and β forms of the sugar may be interconverted, as in glucose.)

fructose

Ribose and its derivative deoxyribose form part of the backbone of the nucleic acids you will meet in Unit 25. Although both these sugars resemble glucose in having a carbonyl group on C-1, they have shorter carbon chains than glucose. Therefore once again, ring closure produces a 5-membered, not a 6-membered, ring.

ribose

* By the way, all the sugars discussed in Units 23 and 24 are D sugars. Sugars of the L configuration (see Units 16 and 17) are rare in living organisms.

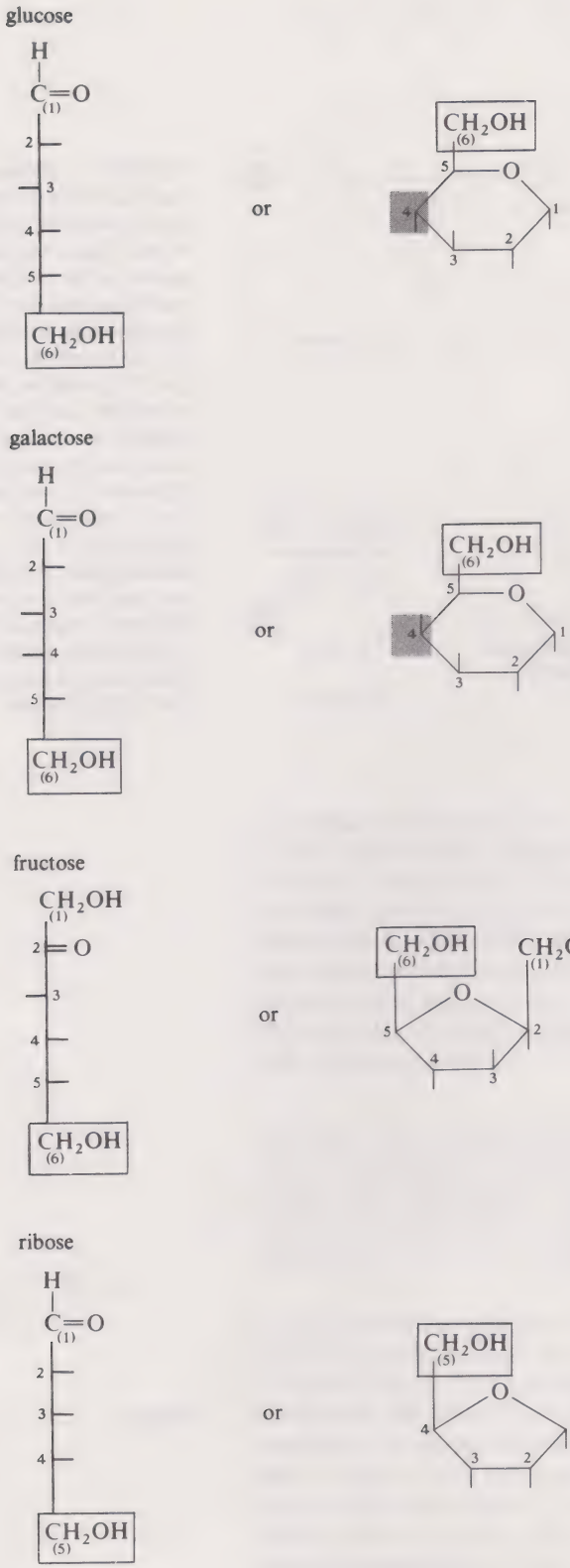


FIGURE 5 Some simple sugars, all in their α-forms. (Remember that the position of OH groups is indicated in straight-chain structures by horizontal bonds sprouting off the carbon backbone, as in Figure 4b. In ring structures these OH groups are indicated by vertical bonds.) You are not expected to remember details of any sugar here except glucose, but you should be aware how the others differ from glucose (compare, for example, the grey shaded areas in galactose and glucose rings). As in Figure 4, the last carbon (C-6 or C-5) is boxed to help you follow the straight-chain to ring transitions.

The following question summarizes what we have just said about sugar structure.

List three ways in which the structure of a simple sugar may be varied to give a chemically distinct molecule.

1 By varying the number of carbons in the chain (for example, glucose versus ribose).

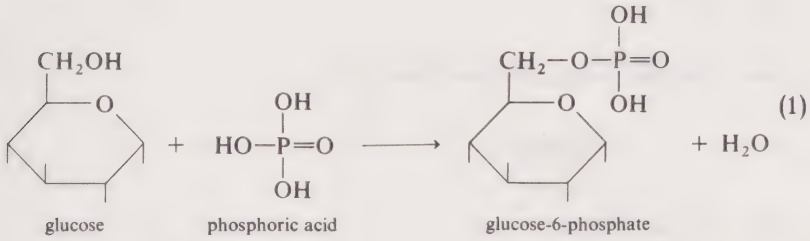
2 By varying the position of the carbonyl group on the chain (for example, glucose versus fructose).

3 By varying the relative orientation of H and OH groups around any carbon other than C-1 (e.g. glucose and galactose, which differ at C-4).

So you see that apparently simple changes can produce quite different structures. In the cell, these may behave very differently.

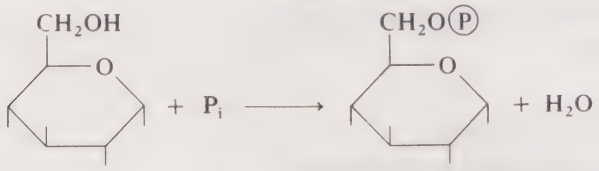
Having described the structure of sugars, we can now turn to some of the more important reactions they undergo in the cell. Esterification by phosphate (the anion* of H_3PO_4, phosphoric acid) is a very common one. Like many other reactions in the cell, this may be visualized as a condensation reaction in which the elements of water are removed from between the two combining molecules. In full, the reaction between a sugar OH (e.g. from glucose) and phosphate may be written as:

inorganic phosphate (P_i)



(1)

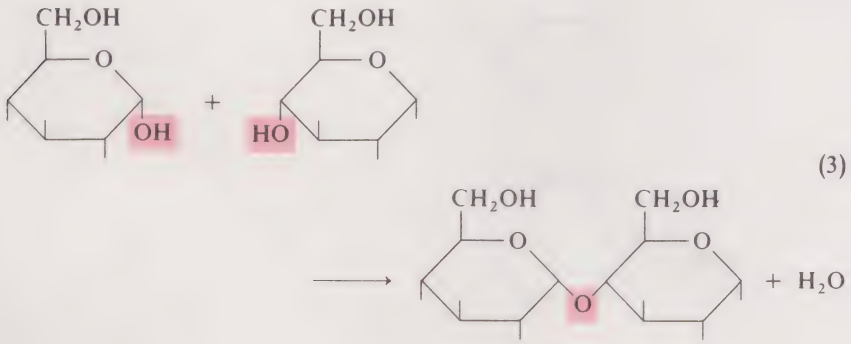
In practice you will often find this abbreviated to:



(2)

phosphate group (PO_3H_2 or Ⓟ)

Another common reaction of sugars in the cell involves the condensation between the OH on C-1 and the OH on another sugar molecule, for example:



(3)

This is how polysaccharides like starch and cellulose are built up, as you will see in Section 10.2.

9.1.1 Objectives of Section 9.1

Now that you have studied Section 9.1, you should be able to:

(a) Number the carbons in both straight-chain and ring formulae of glucose.

(b) Reproduce the ring formulae of α and β-glucose, using the convention depicted in Figures 4d and 4e.

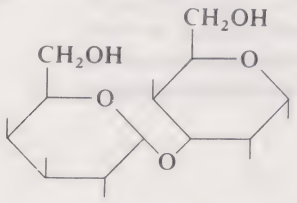
(c) Given the formulae of different sugars, point out whether they vary in the lengths of their carbon chains, the relative positions of H, OH pairs on ring carbons, or the size of their rings.

To test your understanding of these Objectives, try the following SAQs.

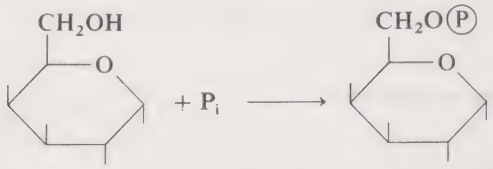
* Because H_3PO_4 has three ionizable OH groups (that is, OH groups that can be dissociated into $-O^-$ and H^+) it forms three different anions. The two most likely to be found in the cell are $H_2PO_4^-$, and HPO_4^{2-}. The term inorganic phosphate (P_i) means simply 'phosphate, ionization state unspecified'.

SAQ 4 (*Objective* (*a*)) Identify the following statements as either TRUE or FALSE.

(i) The following disaccharide is composed of two monosaccharide rings, linked together through their C-1 and C-3 hydroxyls.



(ii) The following equation shows galactose being phosphorylated to galactose-1-phosphate.



SAQ 5 (*Objective* (*b*)) Which is the best description of the difference between the following pairs of sugars (i)–(iii)? Select your answer from the key (more than one description may be chosen).

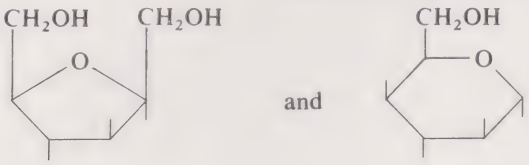
KEY

A The sugars differ in the lengths of their carbon chains

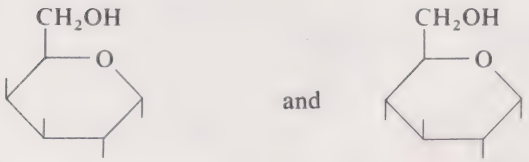
B The sugars differ in the numbers of atoms in their ring structures

C The sugars differ in the relative position of H and OH pairs attached to ring carbons

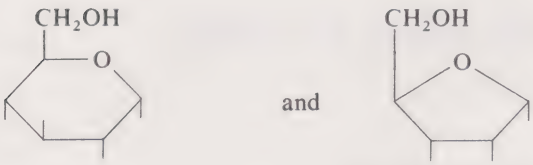
(i)



(ii)



(iii)



9.2 Carboxylic acids, fatty acids and lipids

Study comment This Section introduces several small organic molecules whose reactions (oxidation, reduction, phosphorylation) will be necessary background information for Unit 24. At this stage you should make sure you can recognize these reactions from simple equations. You should also be clear what the terms lipid, neutral fat, carboxylic acid and fatty acid mean.

All these compounds, like the sugars, contain just three elements, C, H and O. The *carboxylic acids*, which you have already met in Units 16 and 17, have the general formula RCOOH. In the simplest examples, R is a short-chain alkyl group; the main constituent of vinegar, for instance, is the carboxylic acid CH_3—COOH or acetic acid. How do the two parts of the molecule, R and COOH, govern the behaviour of carboxylic acids in the cell?

carboxylic acid

The COOH group undergoes all the reactions typical of carboxylic acids (see Units 16 and 17). Like the sugar OH group, it may become linked to phosphate—a common step in chemical transformations of the cell.

The following equation represents the interaction of carboxylic acid and phosphate. (Note that the COOH has been written so as to emphasize the presence of the OH group.)

$$R-\overset{\overset{O}{\|}}{C}-OH + P_i \longrightarrow \text{products} \qquad (4)$$

Use the model in equation 2 (p. 21), which shows the reaction between sugar and phosphate, to give the formula of the products in equation 4.

The products are:

$$H_2O + RCOO\textcircled{P} \text{ (or in full, } R-\overset{\overset{O}{\|}}{C}-OPO_3H_2)$$

Another common reaction of carboxylic acids in the cell is their esterification by the sugar alcohol *glycerol*. This is a 3-carbon compound carrying three OHs, each of which may react with a COOH group (equation 5). The product is known as a *neutral fat*. (Its three R groups may or may not be identical.)

glycerol

neutral fat

$$3R-COOH + \begin{array}{l}CH_2OH\\|\\CHOH\\|\\CH_2OH\end{array} \longrightarrow \begin{array}{l}CH_2O-CO-R\\|\\CHO-CO-R\\|\\CH_2O-CO-R\end{array} + 3H_2O \qquad (5)$$

carboxylic or fatty acid — glycerol — neutral fat

These are the molecules we usually think of as 'fats'. They form a large part of the energy-providing foods in the human diet and, because of their insolubility in water, they can be stored in the body. They also act as insulating layers under the skin of warm-blooded animals.

The term *lipid* includes the neutral fats, the phospholipids (key components of cell membranes) and various steroid hormones and vitamins. Although a vital class of body components, there is unfortunately not space to deal with them in this Course. You should however be aware of their key characteristic—a very poor solubility in water, and a correspondingly high solubility in non-polar solvents such as chloroform.

lipid

The carboxylic acids found in naturally-occurring lipids are known as *fatty acids*. The great majority are long, straight-chain molecules with an even number of carbons. One of the commonest animal fatty acids, for example, is stearic acid*, $CH_3(CH_2)_{16}COOH$. This is a so-called saturated fatty acid, and its R group, $CH_3(CH_2)_{16}-$, contains no double bonds. The unsaturated fatty acids (recommended in the diet of those prone to hardening of the arteries) contain one or more double bonds. They tend to be found in fats of plant rather than animal origin. A typical example is oleic acid, $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$.

fatty acid

Under certain circumstances, animals can adapt to exposure to intense cold by increasing their layers of subcutaneous insulation. This means synthesizing more neutral fat. What small organic molecule components go into such a synthesis?

Glycerol and fatty acid. (Just where the components come from, and how they are put together to form fats, we shall describe in Unit 24.)

The R groups of carboxylic acids in the cell are not always simple long-chain structures like those in the fatty acids. Multi-functional groups may be found attached to COOH residues, as for example in pyruvic acid**, $\boxed{CH_3CO}-COOH$, and fumaric acid $\boxed{COOH-CH=CH}-COOH$. In fumaric acid, the multi-

* As used in the oil-film experiment of Units 10 and 11.

** Notice that throughout this Unit we refer to individual carboxylic acids by the so-called *trivial* names that have been associated with them for many years. They do of course have systematic chemical names, similar to those described in Units 16 and 17, Appendix 1.

functional group (boxed) contains both a double bond and another COOH group. Both acids are exceedingly important in cell chemistry, as we shall see in Unit 24.

You do not need to remember the formula of any particular carboxylic acid, but you should be aware that the functional groups in these and other biological molecules may undergo all the reactions described for groups of this type in Units 16 and 17.

Write down the formulae of the products you would expect to find, for a reaction in which two hydrogen atoms are added to (a) fumaric acid (b) pyruvic acid. (*Note* In the cell the COOH group is *least* likely, of all the functional groups present, to undergo reduction.)

Both starting compounds and products are shown below, with key groups boxed for clarity.

(a) $COOH-\boxed{CH=CH}-COOH + 2H \longrightarrow COOH-\boxed{CH_2-CH_2}-COOH$ (6)

(b) $CH_3-\boxed{CO}-COOH + 2H \longrightarrow CH_3-\boxed{CHOH}-COOH$ (7)

In (a) we have an example of the reduction of a double bond.

In (b) we have an example of the reduction of a carbonyl ($>C=O$) group.

9.2.1 Objectives of Section 9.2

Now that you have studied Section 9.2 you should be able to:

(a) Recognize the meaning of the term carboxylic acid, fatty acid, lipid, neutral fat, glycerol.

(b) Recognize from their formulae, the following compounds: long-chain fatty acid, neutral fat.

(c) Show by means of a simple equation, how neutral fats are formed from fatty acids.

(d) Recognize from formulae and equations the following reactions:

(i) esterification of glycerol by fatty acids

(ii) oxidation (or reduction) by removal (or addition) of H atoms to the carbonyl group in carboxylic acids

(iii) reduction by addition of H atoms across a double bond in carboxylic acids

(iv) reaction between phosphate and carboxylic acid groups.

To test your understanding of these Objectives, try SAQs 6–7.

SAQ 6 (*Objective* (*a*)) Complete the following paragraph, by inserting one of the following words: fatty acid, neutral fat, glycerol, lipid.
By bicycling rather than driving to work, an obese person may use up reserves of ..... These will have been deposited by reaction between ..... and ..... Of these various compounds, those that are insoluble in water, may be described as .....

SAQ 7 (*Objectives* (*b*)–(*d*)) State whether the following equations (i) and (ii) represent:

A Reduction across a double bond in a long-chain fatty acid

B Reduction across a double bond in a carboxylic acid

C Esterification of glycerol by fatty acids

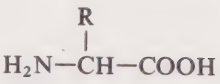
(i) $CH_3(CH_2)_7CH=CH(CH_2)_7COOH \longrightarrow CH_3(CH_2)_{16}COOH$

(ii)
$$\begin{array}{l} CH_2OH + CH_3(CH_2)_{16}COOH \\ | \\ CHOH \ + CH_3(CH_2)_7CH=CH(CH_2)_7COOH \\ | \\ CH_2OH + CH_3(CH_2)_{16}COOH \end{array} \longrightarrow \begin{array}{l} CH_2OCO(CH_2)_{16}CH_3 \\ | \\ CHOCO(CH_2)_7CH=CH(CH_2)_7CH_3 \\ | \\ CH_2OCO(CH_2)_{16}CH_3 \end{array}$$

9.3 Amino acids

Study comment The important point of this Section is to be able to reproduce the general formula for an amino acid, and to be aware of the great range of different amino acid sidechains that may be found in proteins.

The bulk of the nitrogen in the cell is found in amino acids linked together as proteins. The general formula of an amino acid (see Units 16 and 17, Section 4.2) is $R{-}CH(NH_2){-}COOH$ or as shown in the margin. Since the majority of amino acids are combined into proteins*, their carboxylic acid and amino groups are not available for reaction: they have been used up in peptide bond formation, as we shall shortly see. What are always prominent in proteins are the amino acid sidechains, the R groups which give to each amino acid its individuality. There are about twenty different kinds of R group in naturally occurring amino acids, and these are listed in Table 5. You are expected to recognize only two of these—glycine and alanine. But you should be clear about the kind of variety that can be found. Note particularly in Table 5 the following types of sidechains: those that carry positively-charged groups (for example, lysine and arginine), those that carry negatively-charged groups (for example, glutamic and aspartic acids), those that are small and inert (for example, glycine and alanine), those that are large and bulky (for example, phenylalanine, tyrosine and tryptophan), and those that have special reactive groups (for example, SH in cysteine, OH in tyrosine, threonine and serine).



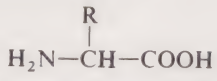
amino acid sidechain (R group)

TABLE 5 Amino acids and proline**

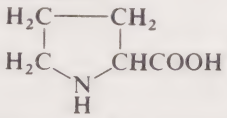
Amino acid	Abbreviation	Formula of R group
alanine	Ala	CH_3
arginine	Arg	$H_2N{-}C(=NH_2^+){-}NH{-}(CH_2)_3$
asparagine	Asn	$CONH_2{-}CH_2$
aspartic acid	Asp	$COO^-{-}CH_2$
cysteine	Cys	$SH{-}CH_2$

Table 5 is continued overleaf

** The majority of amino acids have the formula



where R may be any of the 19 alternatives shown in this Table. The exception is *proline*, which has no free NH_2 group. Its formula is



and it is called an *i*mino acid. Note that charged groups are indicated in pink in the Table. Reactive OH and SH groups are shaded grey.

* Small amounts of amino acids are also found in hormones. *Insulin* is a small protein (relative molecular mass 6000) and *thyroxine* is very much smaller—simply an iodinated form of the amino acid tyrosine.

TABLE 5 (continued)

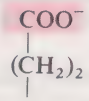
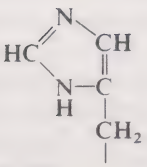
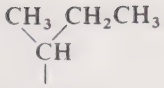
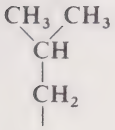
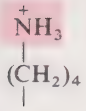
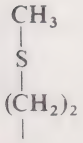
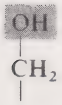
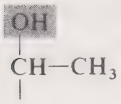
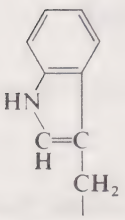
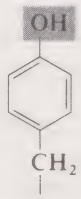
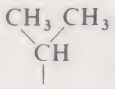
Amino acid	Abbreviation	Formula of R group
glutamic acid	Glu	
glutamine	Gln	
glycine	Gly	
histidine	His	
isoleucine	Ile	
ieucine	Leu	
lysine	Lys	
methionine	Met	
phenylalanine	Phe	
serine	Ser	
threonine	Thr	
tryptophan	Trp	

TABLE 5 (continued)

Amino acid	Abbreviation	Formula of R group
tyrosine	Tyr	
valine	Val	

All proteins, unlike the polysaccharides and fats we have discussed so far, contain nitrogen as well as the elements of carbon, hydrogen and oxygen. Some, like the cytochromes you will meet in Unit 24, and the oxygen-carrying protein haemoglobin, also contain an atom of iron. Other metals commonly associated with proteins are zinc, calcium and manganese. In addition, sulphur is found in proteins containing the amino acids methionine and cysteine.

9.3.1 Objectives of Section 9.3

Now that you have studied Section 9.3, you should be able to:

(a) Reproduce the general formula for an amino acid, and the specific formulae for glycine and alanine.

(b) Given the formula of an amino acid, state whether it is small or large and bulky, negatively or positively charged, and whether or not it contains reactive SH or OH groups.

To test your understanding of this, try SAQ 8.

SAQ 8 (*Objective* (*b*)) Describe the following statements as TRUE or FALSE.

(i) Tryptophan has a small, positively charged sidechain.

(ii) Alanine has a small, neutral sidechain with no reactive groups on it.

(iii) Cysteine is the only amino acid with a free SH group in its sidechain.

(iv) Aspartic acid, when combined in a protein, still has a free COOH group.

9.4 Purine and pyrimidine bases and nucleotides

Study comment The role of these compounds in nucleic acids will be dealt with much more fully in Unit 25. However, the structure of nucleotides will be essential knowledge for Unit 24. Figure 7 summarizes the most important points of this Section.

Like amino acids, purine and pyrimidine bases contain nitrogen as well as carbon, hydrogen and oxygen. Quantitatively, they make up only a very small proportion of the components of the cell, but their influence on cell life is enormous. In Unit 25 you will come across them as the key message-encoding components of the hereditary material of the gene. In Unit 24 you will find that one of the purines—adenine—is part of adenosine triphosphate (ATP), a substance prominent in numerous chemical transformations of the cell.

The general formula for a purine is shown in Figure 6. Both adenine and guanine are common examples, while the common pyrimidines are cytosine, uracil and

thymine. The term *base* is used to mean 'pyrimidine or purine'*. All these are shown in Figure 6—the only parts you need to be familiar with are the purine and pyrimidine rings on the extreme left.

purine and pyrimidine bases

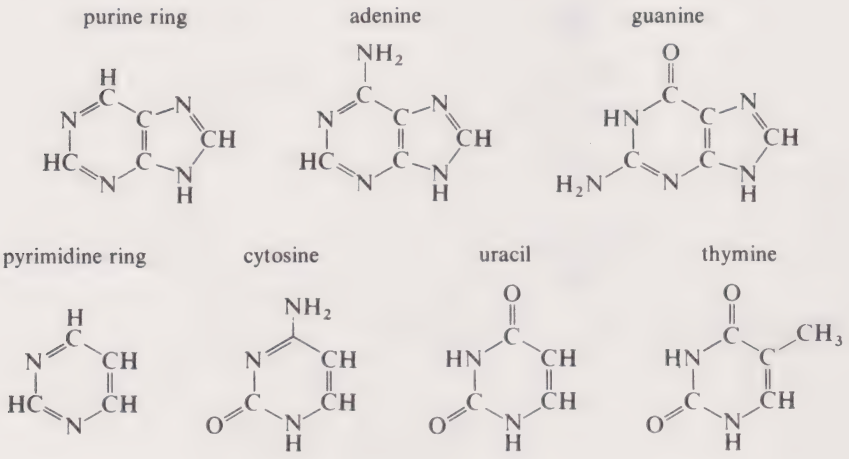


FIGURE 6 Purine and pyrimidine bases.

Neither purines nor pyrimidines are particularly reactive molecules and are nearly always found attached to phosphate via a 5-carbon sugar, ribose** or deoxyribose, that is as [base—sugar—phosphate]. This three-part molecule is known as a *nucleotide*. In its most reactive form, the nucleotide has not one but three phosphates (Figure 7). It is the outermost phosphate group that most often takes part in chemical transformations of the cell: the purine or pyrimidine base is concerned mainly with direction and control. The most common reactions of ATP for example, involve removal of the terminal phosphate group to give adenosine diphosphate or ADP. (This important reaction is described more fully in Unit 24.) Where nucleotides are linked together in a chain to form nucleic acids (see Unit 25), the *two* outermost phosphates are removed.

nucleotide

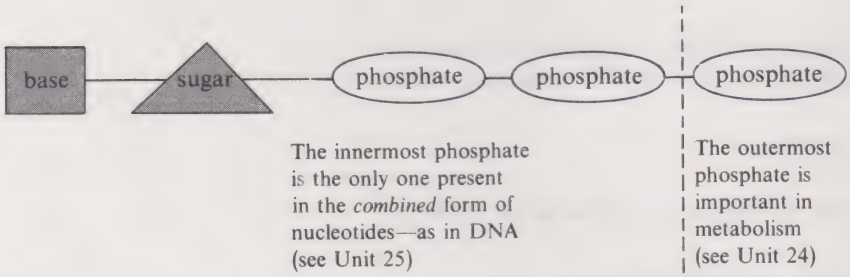


FIGURE 7 Structure of a nucleotide.

9.4.1 Objective of Section 9.4

Now that you have studied Section 9.4, you should be able to:

(a) Recognize the general formula of purine and pyrimidine bases, and show diagrammatically how they are linked together with phosphate and sugar to form a nucleotide.

To test your understanding of this Objective, try SAQ 9.

SAQ 9 (*Objective* (*a*)) Which of the following components A–D may be described as nucleotides?

A Lysine—glucose—phosphate

B Adenine—phosphate—glucose

C Adenine—glucose—phosphate

D Adenine—ribose—phosphate

* It refers to the fact that, when in solution on their own, these compounds do act as bases (see Unit 14).

** The formula of ribose is given in Figure 5.

9.5 Objectives of Section 9

Now that you have read the whole of Section 9, you should be able to:

(a) List the four main classes of compound found in the cell, the elements they contain and the small organic molecules of which they are built up.

(b) Recognize the meaning of all the following terms: sugar, monosaccharide and polysaccharide, carbohydrate, lipid, fatty acid, neutral fat, glycerol, amino acid sidechain, nucleotide, long-chain fatty acid.

(c) Distinguish the following molecules from their formulae: monosaccharide (i.e. sugar), fatty acid, glycerol, amino acid, nucleotide.

To test your achievement of these Objectives, try SAQs 10–12:

SAQ 10 (*Objective* (*a*)) Complete Table 6 by putting a tick in each column to show which elements you would expect to find in each kind of compound.

TABLE 6 Elements found in different classes of cell chemicals.

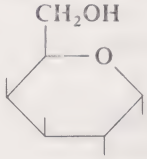
Elements	Classes of cell chemicals			
	Protein	Polysaccharide	Fat	Nucleotide
carbon				
hydrogen				
oxygen				
nitrogen				
sulphur				
phosphorus				
iron				
manganese				

SAQ 11 (*Objectives* (*a*) *and* (*b*)) Table 7 lists some common foodstuffs, and the classes of macromolecule they contain. (Fats are included here because, like these macromolecules, they are formed by condensation reactions between small organic molecules.) You are asked to fill in the last column by listing the small organic molecules that could be obtained by breakdown of the nutrient molecules. Select your answers from the following: amino acid, fatty acid, sugar, glycerol.

TABLE 7 Components of macromolecular foodstuffs. (For use with SAQ 11.)

Food	Main macromolecular component	Main small organic components
milk	fat protein water	
fish	protein	
gelatine used for setting 'jellies'	protein	
bread	starch	
lettuce leaf	cellulose	
butter	neutral fat	
cooking oil	neutral fat	
meat	protein	
porridge	protein and carbohydrate	

SAQ 12 (*Objective* (*c*)) State whether the following formulae represent nucleotide, sugar, amino acid or fatty acid:

(i) $C_6H_{12}O_6$ or 

(ii) $CH_3(CH_2)_{18}COOH$

(iii) $CH_3CHCH(NH_2)COOH$ (the CH_3 group is attached to the second carbon)

10 Macromolecules in the cell

Study comment This is an important Section, in which you should note particularly the way globular proteins fold, producing specific recognition sites on their surfaces. For the last part of this Section you will require the stereoviewer and Filmstrip 23.3.

10.1 Primary structure

The small molecules we have just described do not exist in the cell to any great extent on their own. The great majority are linked together into macromolecules, with relative molecular masses ranging from one thousand to several millions. Each macromolecule may be imagined as a long string of sausages, joined to one another by a covalent bond (the bits of skin between the sausages). The sausages represent the component small molecules of the macromolecule—amino acids in proteins, sugars in polysaccharides, and nucleotides in nucleic acids. In the cell, the string of sausages is built up by adding molecules step by step to one end of a growing chain (see Figure 8). In all three classes of macromolecule, the covalent bond which links the oncoming molecule to the rest of the chain is formed by a reaction in which the elements of water are removed.

FIGURE 8 Building up a macromolecule. The black sphere represents an oncoming small molecule being added to the end of the growing macromolecular chain.

Where the macromolecule is a protein, the reaction takes place between a COOH group at the end of the chain, and an NH_2 group on the oncoming amino acid (see Figure 9). The resulting link is known as a *peptide bond*. (A string of amino acids is known as a polypeptide. This is why proteins are sometimes referred to as polypeptide chains.)

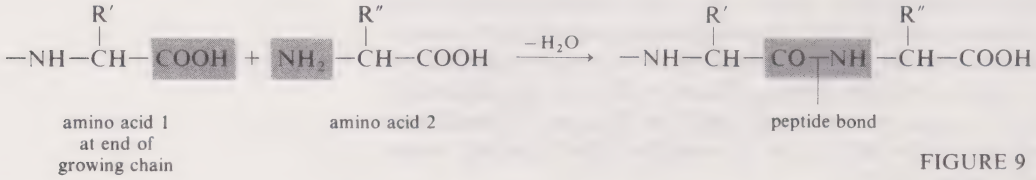


FIGURE 9 Peptide-bond formation.

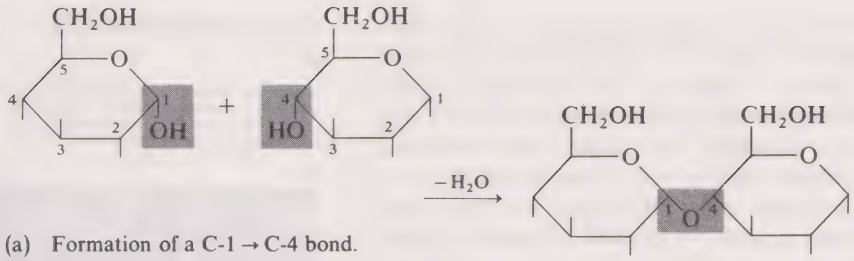
Where the macromolecule is a polysaccharide, the covalent bond is formed between OH groups on adjacent sugar rings, as shown in Figure 10a. Although the first sugar, drawn on the left, always combines through the OH group on C-1, the second sugar may combine through the OH on C-4 (as shown in Figure 10a), C-6 (as shown in Figure 10b), or other OH groups.

Where the macromolecule is a nucleic acid, the bond is formed between the different nucleotides, as you will find in Unit 25.

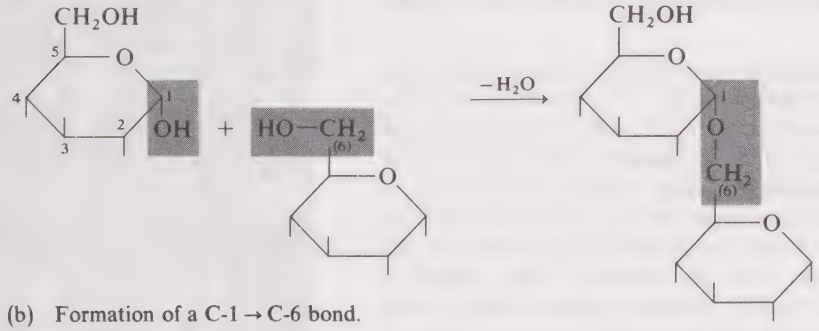
primary structure

The *primary structure* of a macromolecule is concerned partly with the *nature* of the small-molecule components that go to build it up, and partly with the *order* in which these components are strung together. Primary structure, directly or indirectly, is under control of the genetic material of the cell, and it has far-reaching consequences, both for the shape of the finished macromolecule and for what it does in the cell.

Some macromolecules have highly irregular primary structures, for instance A—C—E—D—B—F—A—D—D—. For a protein, a specific example might be: glycine—proline—arginine—etc. Other macromolecules have highly repetitive primary structures (glucose—glucose—glucose—glucose... or glycine—proline—



(a) Formation of a C-1 → C-4 bond.



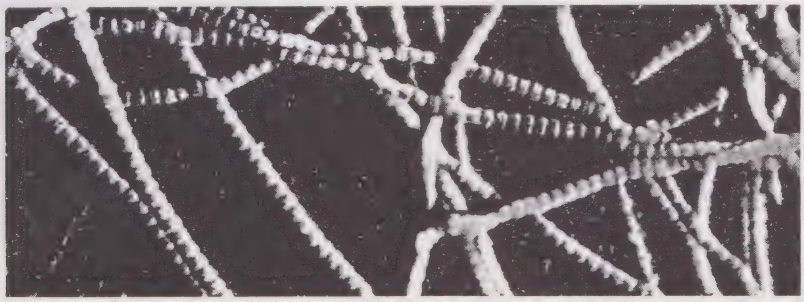
(b) Formation of a C-1 → C-6 bond.

FIGURE 10 Linking together sugar residues in a polysaccharide. Grey shadii emphasizes the groups involved in bond formation.

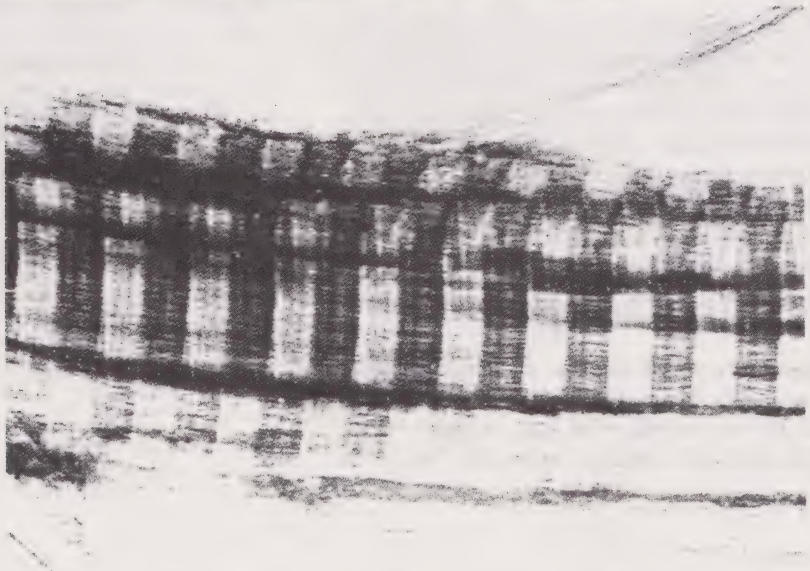
alanine—glycine—proline—alanine... etc.). A highly repetitive primary structure is associated with a support role for the macromolecule, as in the fibrous proteins, or a support or food-storage role, as in the larger polysaccharides.

10.2 Support and food-storage macromolecules

The support molecules form extended unbranched chains which can pack side by side with similar molecules to give fibres of considerable strength. The *fibrous proteins* may be exemplified by *collagen* (see Figure 11), one of the major constitu-



(a)



(b)

(c)

FIGURE 11 The structure of collagen, a fibrous protein. (a) Electron micrograph at ×30 000 magnification, showing individual fibres. (b) Electron micrograph at ×300 000 magnification, showing a single fibre taken from (a) in ten times more detail. (c) Molecular diagram of the smallest unit in (b), a *fibril* formed from three intertwined protein chains.

ents of animal connective tissue*. Its primary structure is largely a repeat of the tripeptide sequence (glycine—proline—X), where X is often alanine. A typical *fibrous polysaccharide* is *cellulose*, shown in Figure 12. This too has a regular primary structure—a string of glucose molecules joined through their C-1 and C-4 carbons (as in Figure 10a). As in collagen, this regular and unbranched primary structure permits it to assemble together with other molecules into a very tough fibre. Try breaking a thread of cotton—which is almost pure cellulose—and you can test this fibrous strength for yourself. In nature, cellulose forms the main supporting framework of the cell walls in higher plants (Figure 12c).

Another polysaccharide of highly repetitive primary structure is *starch*. Like cellulose, it is composed solely of glucose** molecules, but not all of these are linked between C-1 and C-4. Occasional branch points occur, where the linkage is between C-1 and C-6 (Figure 10b). As a result, the molecule resembles a bush rather than a fibre (Figure 13). Adjacent molecules cannot reinforce one another by parallel stacking as in cellulose, and starch is no good as a support molecule. However, its open bush-like structure is ideal for packing large numbers of glucose molecules into a small space and yet allowing ready access to polysaccharide-hydrolysing enzymes. These chop glucose residues from the ends of the branches, ready for use in energy-producing reactions of the cell. Starch is the main food reserve of higher plants. The higher animals use *glycogen*, another glucose polymer with very similar branching structure.

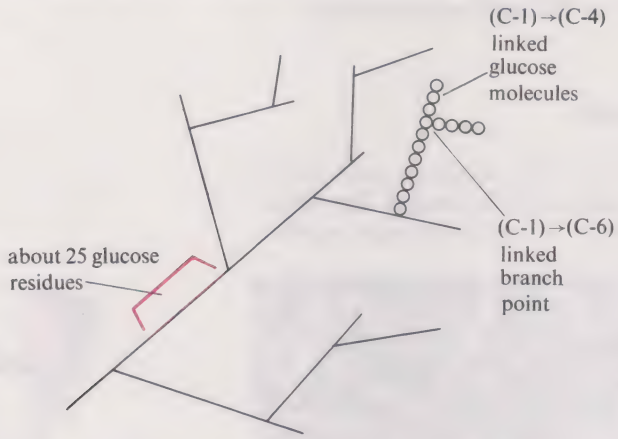


FIGURE 13 Structure of starch, a food-reserve polysaccharide.

10.3 Globular proteins and specific recognition sites

We return now to those macromolecules where primary structure is not repetitive but highly irregular. Although it is now recognized that numerous short lengths of polysaccharide come into this category—and play a vital role in the control of body chemistry—we shall concentrate here on the proteins. (The significance of the irregular primary structure of nucleic acids will become apparent in Unit 25.) Proteins of irregular primary structure have quite a different shape from the fibrous proteins. They fold up, during synthesis, into compact 'globules' like balls of string (see Figure 14). Hence they are known as *globular proteins*. Many of the more interesting proteins come into this category. They include the biological catalysts known as *enzymes*, the oxygen-carrying proteins such as haemoglobin, and the protein hormones such as insulin and growth hormone.

Despite its irregularity, the primary structure of a globular protein is precisely defined. All molecules of a given protein have the same length and the same amino acid sequence. This contrasts with fibrous proteins like, say, collagen, where molecules may vary in overall length and in the nature of every third residue in the chain.

FIGURE 12 Structure of cellulose, a fibrous polysaccharide.
(a) C-1 → C-4 linked sugars (designated ○ form a *single chain*.
(b) Chains are stacked parallel to form a *fibre*.
(c) Layers of fibres form a *cell wall*. (Magnification ×20 000.)

globular protein

enzymes

* Connective tissue 'connects' many of the vital organs of the body. It underlies muscle tissue, for example, and can be seen in gristle and the stringy bits of meat.

** The glucose residues in starch and cellulose differ at C-1; starch is made up of α-glucose and cellulose of β-glucose (see Figure 4).

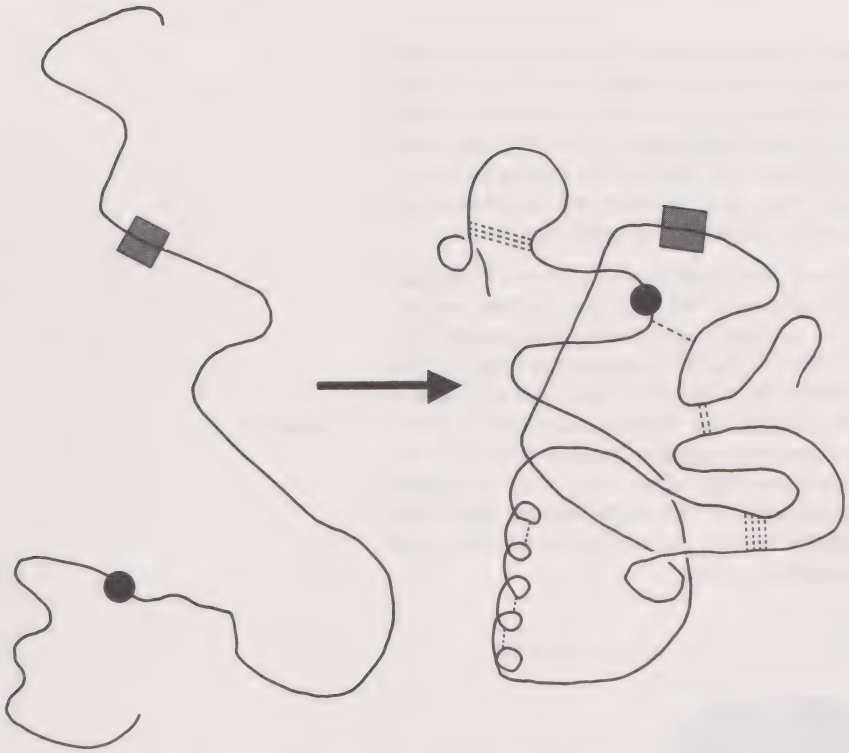


FIGURE 14 Formation of a globular protein by folding of the protein chain. The symbols ▩ and ● represent amino acids situated at different points in the primary structure. Dashed lines indicate some of the many weak bonds holding together different lengths of the folded chain.

As we have just seen, primary structure determines the overall shape of a macromolecule (for example, whether it is bush-like, fibre-like or globular) and it is this shape that determines its biological role. For the support and food-storage molecules, small variations in primary structure can be tolerated quite happily. The same is not true of globular proteins, where the biological role of the folded-up molecule depends critically on the complete reproducibility of its surface geometry. Let us look now at the way this surface geometry is produced.

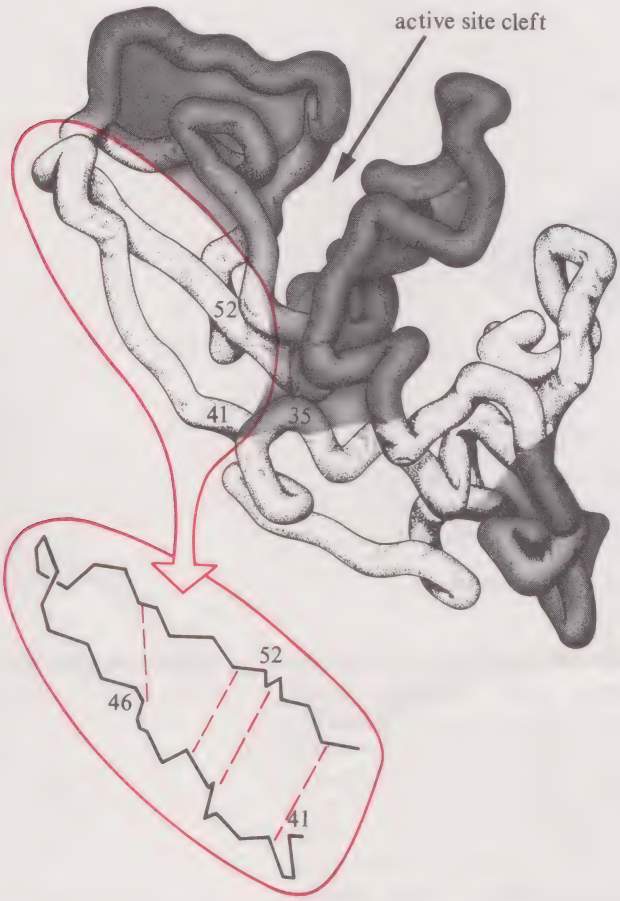


FIGURE 15 Structure of lysozyme, a globular enzyme protein. (This protein catalyses destruction of the polysaccharide components in bacterial cell walls. It is found in many body fluids, including tears, and acts as an internal bacteriocide.)
(a) Folding pattern of protein chain. Although this looks like a piece of folded-up wire, to oncoming molecules it presents a precisely defined surface, as indicated by the grey shading.
(b) Details of one section (outlined in red in (a)), showing stabilizing weak bonds as red dashed lines. Numbers refer to positions of amino acids in the primary structure.

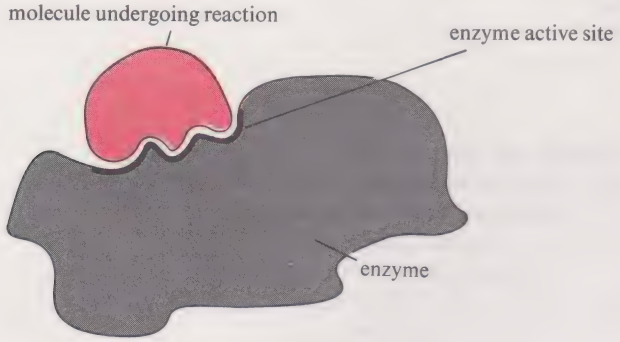
When the protein chain folds in the cell, as shown in Figure 14, it does so in a *highly exact and strictly reproducible way*. Every single molecule of the protein lysozyme for example (shown in Figure 15a) will have this same strangely convoluted folding pattern. The folding pattern is known as *higher-order structure*. To

higher-order structure

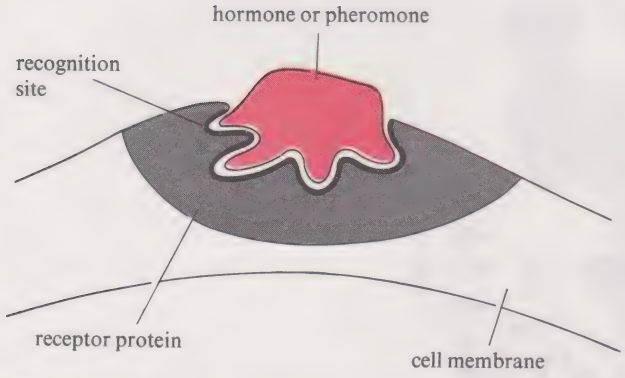
see how it affects the surface geometry, concentrate now not on the polypeptide chain (the individual strands of 'string' inside the protein) but on the overall shape of the whole 'ball' of string. If you took a plaster cast on the surface of the ball and looked at it under exceedingly high magnification, it would look rather like the wrinkled surface of a walnut (Figure 15). Because the folding pattern of the protein chain is precisely defined, each protuberance and depression will reoccur in just the same position on *every* molecule of a particular protein.

The need for such reproducibility is best illustrated by the enzymes, biological catalysts of immense importance in cell chemistry. We shall describe their properties in more detail in Section 11. For the moment it is necessary to emphasize only one point—enzymes show enormous selectivity in the reactions they catalyse. The mechanism behind this selectivity involves the precise three-dimensional shape of the *active-site* region of the enzyme surface (see Figure 16a). This is where molecules taking part in the enzyme-catalysed reaction become bound to the surface and undergo reaction. Binding can take place only if the oncoming molecule has a surface *exactly complementary* to that of the enzyme active site. Other molecules may brush up against the surface, but will not fit into the active site and therefore there will be no catalytic reaction.

active site



(a)



(b)

FIGURE 16 Specific recognition sites on the surface of globular proteins (shaded grey). Heavy black lines indicate areas that are complementary in shape to oncoming molecules (shaded pink).
(a) Enzyme active site.
(b) Recognition site on membrane-bound receptor protein.

The enzyme active site is just one example of a *specific recognition site* built into the higher-order structure of a macromolecule. Other examples include membrane-bound receptor proteins for hormones and pheromones (Figure 16b). Hormones, for example, pass from one tissue to another in the bloodstream of higher animals, or diffuse between the cells of higher plants. They are picked up by the target cells for which the chemical message is intended, by binding to interaction sites on receptor proteins located in the cell membrane (or sometimes, within the cytoplasm). Muscle cells in the leg, for instance, can respond to the hormone insulin secreted into the bloodstream by the pancreas (perhaps a metre away) at a concentration of only a few molecules per mm^3 of blood. Other cells, like those of kidney and liver, have no insulin receptors in their cell membranes and therefore do not respond to it. At the *molecular* level, this communication network relies on interaction between the hormone and a complementary binding site in

specific recognition site

the surface of the receptor molecule. It makes understandable the high specificity of the hormones that you studied at the *cellular* level in Unit 22.

In a similar way, an insect's response to pheromones depends on a specific binding site built into the higher-order structure of a receptor protein on the antenna. In Units 16 and 17 you saw how even minor variants of the true pheromone failed to elicit any response from the insect. These pheromones presumably have the wrong shape to fit into the binding site.

All these molecular recognition mechanisms are determined by the higher-order folding pattern of the protein chain. However, what ultimately determines folding pattern is the sequence of amino acids in the chain. Slightly different primary structures will not fold to the same higher-order structure. (This is an essential point and applies to all classes of macromolecule.) This may be disastrous for the biological functioning of the protein, particularly if the change affects the shape of its specific recognition sites. As we shall see in more detail in Unit 25, the primary structure of a protein is directly controlled by the genetic material of the individual.

10.4 Weak bonding and higher-order structure

If higher-order structure is so important in the biological role of a macromolecule, you might well ask how it is stabilized. What is it that holds adjacent strands of the backbone chain together? The answer is—*weak bonds*. Three kinds of weak bonding are found in the cell: hydrogen bonds (see Units 16 and 17), hydrophobic bonds* (based on the London dispersion forces described in Units 16 and 17) and ionic bonds. These last result from the mutual attraction of ions of opposite charge (for example, between negatively charged aspartic acid and positively charged lysine; see Table 5 for amino acid formulae).

weak bonds

Although individually these bonds are all much weaker than the covalent bonds linking individual residues in the chain, there are a great many of them. Under biological conditions, therefore, the higher-order folding pattern is stable.

> Given this information about weak bonds, can you now see why biochemists avoid subjecting their cell extracts to high temperatures?
>
> Weak bonds require far less energy for their disruption than covalent bonds, and so the higher-order structure of delicate macromolecules in the cell extract would break down at high temperatures.

Extremes of pH have the same effect on weak-bonded structures as high temperatures. An ionic bond involving a COO^- group on aspartic acid, for example, would cease to exist if the pH were lowered to the point at which COO^- became COOH.

This loss of higher-order structure in a macromolecule by disruption of the weak bonds which hold it together, is a phenomenon known as *denaturation*. It is an unfortunate—though not infrequent—hazard in working with these molecules. In the Home Experiment at the end of this Unit, you will be asked to denature egg-white protein deliberately, using much the same method as you would in boiling an egg.

denaturation

10.5 Stereopictures of proteins

Study comment For this Section, you will need the stereoviewer, and Filmstrip 23.3, which shows the following:

Frame 1 A fibrous protein.

Frame 2 A globular protein, lysozyme.

Frame 3 An enzyme, lactic dehydrogenase.

* Hydrophobic ('water-hating') bonds are formed when non-polar atoms or groups of atoms interact, squeezing out any hydrophilic ('water-loving') polar groups that may lie between them. If you watch the way cream droplets coalesce on top of a (preferably rather cool!) cup of coffee, you have an example of hydrophobic bond formation.

Three-dimensional shape is clearly extremely important in the biological role of macromolecules. Yet so far we have restricted our descriptions to two-dimensional representations of three-dimensional structures. The stereoslides introduced here give a far truer picture of the protein molecule as it really exists.

The following points come over particularly forcefully in these slides, which you should now study:

Filmstrip 23.3

1 *Frame 1* Fibrous proteins are not flat, but are *specifically kinked* in a way that is stabilized—like the higher-order structure of globular proteins—by weak bonding between adjacent molecules. Frame 1 shows the fibres of a structural protein like silk. You can see the parallel polypeptide chains running vertically up the slide. The *orange* section delineates the extent of one amino acid residue, —NH—CHR—CO—. Hydrogen bonds running at right angles between adjacent molecules are shown in *red*.

2 *Frame 2 In globular proteins, amino acids that are quite distant in primary structure may be brought close together by the higher-order folding pattern.* On the right-hand side of Frame 2 you can see a sausage model of the backbone chain of lysozyme. (This enzyme is depicted also in Figure 15.) This enzyme is responsible for catalysing the puncturing of holes in the protective polysaccharide coat that surrounds most bacteria. (It occurs, for example, in tears, where it keeps the eyeball surface disinfected.) On the left of the sausage model are two parallel, purplish-white lengths of chain. Although they lie so close in the final folding pattern of the molecule, and indeed are held together by hydrogen bonding (shown red in Frame 1), the lower ends of these purplish-white sections contain amino acids from different parts of the extended chain—residues 41 and 54, to be precise (see Figure 15b).

3 *The enzyme active site is truly a cleft in the molecule.* Look first at Figure 15a, where the active site is arrowed, and then at the views in the sausage model of Frame 2. The detailed model of lysozyme on the *left* of the slide shows individual atoms in the molecule. You need *not* study this in detail, but should appreciate that the active-site cleft is lined with a whole range of different amino acid sidechains—the yellow ones depict sulphur-containing residues, the red ones carry charges (for example, aspartic acid) while the purplish-blue ones are the hydrophobic (water-insoluble) residues of amino acids like tryptophan and alanine.

4 *Frame 3 The active site may provide a specific fit, through matching complementary surfaces, to quite large molecules.* The enzyme depicted here, lactic dehydrogenase, features again in Unit 24. Here it is shown with one of the molecules it interacts with, the nucleotide nicotinamide–adenine dinucleotide (NAD), which will also be described in Unit 24. The bright blue balls lying flat, near the top of the molecule, are the individual atoms of NAD. The white balls show the individual atoms of the protein backbone

(The R groups, that is, the amino acid sidechains, are omitted in this model.)

10.6 Summary of Section 10

1 The primary structure of a macromolecule describes the *nature* of its component small molecules, the *kind of covalent links* between them (for example, C-1 → C-4 or C-1 → C-6 in polysaccharides) and the *order* in which they are strung together.

2 Higher-order structure describes the way in which the string of covalently-linked small molecules folds up in the cell. This folding pattern is essential for the biological activity of the molecule. It is stabilized by weak bonds and is therefore readily disrupted by heat and extremes of pH.

3 Molecules of different primary structure fold to different higher-order structures. Therefore small changes in the amino acid sequence of a globular protein may disrupt the structure of its specific recognition sites and hence destroy its biological function.

4 Molecules with regular primary structures tend to assume either bush-like or fibrous shapes. These fit them for their respective roles as *food-storage* or *support* molecules.

5 Globular proteins have irregular primary structures, and fold into globules with highly convoluted surfaces. This produces *specific recognition sites* on the surface of the protein. These sites have shapes complementary to the molecules with which they interact. Examples of specific recognition sites are enzyme active sites and receptor binding sites for hormone or pheromone.

10.7 Objectives of Section 10

Now that you have studied Section 10, you should be able to:

(a) Explain what is meant by primary structure in polysaccharides, proteins and nucleic acids.

(b) Describe in general terms (without formulae) the primary structures of support and food-storage polysaccharides, and support and catalytic proteins.

(c) Explain how the biological role of a globular protein is influenced by both its primary and its higher-order structure.

(d) Explain in terms of weak bonding and higher-order structure, why proteins lose their biological activity at high temperatures and extremes of pH.

To test your understanding of these Objectives, try SAQs 13–15.

SAQ 13 (*Objectives* (*a*), (*c*) *and* (*d*)) Identify the following statement as either TRUE or FALSE.

Disrupting the weak bonds of a macromolecule will destroy its higher-order structure but not its primary structure.

SAQ 14 (*Objectives* (*a*)–(*c*)) Identify the following statements as either TRUE or FALSE.

(i) Chemical analysis of two recently isolated polysaccharides revealed that both were polymers of glucose and ribose sugars; this means that their primary structures will be identical.

(ii) Food-storage polysaccharides tend to have regular primary structures which enable them to pack together as long, extended chains.

(iii) The primary structure of protein P was found to be $(\text{glycine—alanine—proline})_n$: this suggests it is more likely to be a support protein than an enzyme.

(iv) Support polysaccharides tend to have regular primary structures with occasional branch points.

SAQ 15 (*Objectives* (*c*) *and* (*d*)) Would you expect an adrenalin-binding protein, which has been isolated from the cell membrane by a technique involving strongly acid solutions, to retain its ability to bind adrenalin? Give a reason for your answer.

11 Enzymes

Study comment This Section contains essential background information for Unit 24. It links the *structure* of one particular type of macromolecule, the globular proteins, with one of their *functions*—that of enzymic catalysis. Some of the information in this Section is illustrated and further expanded in the biochemistry Home Experiment, which forms the bulk of Section 11.7. The experiment takes about two hours to do, and is closely integrated with the rest of the Section. Objectives and SAQs occur in Section 11.8, after the Home Experiment Notes.

11.1 General properties of enzymes

Practically every chemical reaction taking place in the body is catalysed by an enzyme. It is therefore important to remember that however miraculous their effects may seem, enzymes do not contravene the ordinary laws of physics and chemistry. Like all catalysts they have the following restrictions:

1 They cannot catalyse a reaction that would not otherwise take place. They can only speed up reactions that are already happening. (It may *seem* that the reaction occurs only on addition of enzyme, but this is because the previous, uncatalysed reaction rate was extremely slow.)

2 Enzymes cannot change the equilibrium position of a reaction (see Unit 15, Section 5.3).

3 Enzymes are not used up during the reaction. Therefore just a few molecules of an enzyme may be sufficient to catalyse the interconversion of large numbers of reacting molecules.

In all these ways, enzymes are no different from other catalysts. However, they have two outstanding characteristics seldom found in other catalysts. These can be seen by comparing an enzyme-catalysed reaction that normally takes place in the cell, with the same reaction carried out in a test-tube, in the presence of non-enzymic catalysts.

1 They are capable of extreme selectivity (specificity) in the reactions they catalyse.

2 They catalyse reactions under very mild conditions (that is, without the need for extreme acidity, temperature, strong oxidizing agents, etc.).

By the end of this Section you should have several examples of these two characteristics.

11.2 Effect of temperature and pH on enzyme-catalysed reactions

optimum pH

Each enzyme operates most efficiently at a particular pH. This *optimum pH* varies with each enzyme, depending on the nature of amino acid groups in the active site. For example, several reactions involve catalysis by negatively charged groups like COO^-. Below a certain pH this will exist mainly as COOH (see Unit 14) and therefore little catalysis will take place. Whatever the precise value of the pH optimum, it seldom lies outside the range pH 4–9*. Below and above these values the delicate weak-bonded structure of the enzyme is liable to be destroyed.

As a result of this balance between the pH required for catalytic activity, and the pH which leads to denaturation, many enzymes show bell-shaped curves when their catalytic activity is plotted against pH. A typical curve is shown in Figure 17.

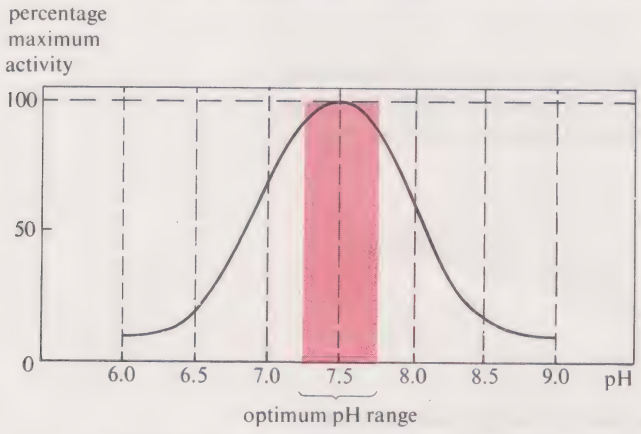


FIGURE 17 Effect of pH on enzyme activity.

optimum temperature

All chemical reactions have a so-called *optimum temperature* but with enzymes this term is rather misleading, because it depends critically on the duration of the

* Pepsin, which you will study in the Home Experiment for this Unit, is an exception.

reaction. For example, very rapid reactions might be observed *in vitro* even at 70 °C, but only for the few seconds before the enzyme structure is destroyed by heat. When reactions are taking place *in vivo* over prolonged periods, the optimum temperature is usually around that of the organism, 37 °C for mammals. (Surely no coincidence, but an example of the efficiency of natural selection!)

Studies on the temperature dependence of enzyme-catalysed reactions give a clue to the way enzymes operate so successfully under mild conditions. Like all catalysts, they are remarkably effective in lowering the *activation energy*, (E_a) of the reactions they catalyse. Figure 18 demonstrates this, by comparing the reaction profiles of an un-catalysed and an enzymically-catalysed reaction.

activation energy (E_a)

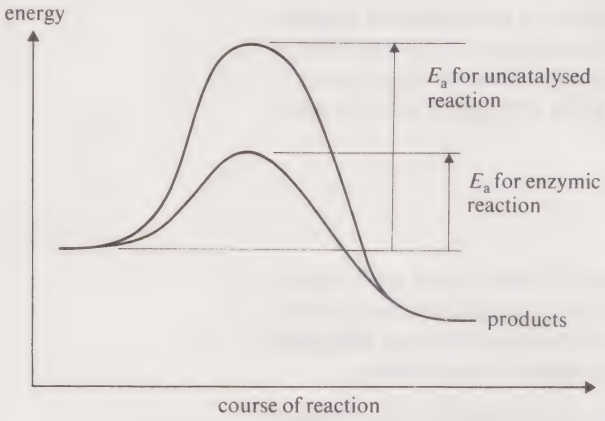


FIGURE 18 Activation energy E_a.

11.3 Enzyme specificity

One of the most distinctive features of enzymic catalysis is specificity. To understand this concept we must return to the active site, the region on the enzyme surface where catalysis takes place. Any molecule that fits into the active site and becomes chemically altered by the enzyme is a *substrate*. In the pepsin Home Experiment for example, the reaction you will study is the breakdown of egg-white protein to small peptides, and the substrate is therefore egg-white protein.

substrate

The fit between enzyme and substrate at the active site is crucial to the whole catalytic reaction. Only if the substrate fits absolutely correctly—like a key fitting into a lock—will the parts to be chemically modified be correctly aligned with respect to catalytic groups in the active site (see Figure 19). These catalytic groups are the amino acid sidechains (the R groups of Table 5) that have been brought into the active site by folding of the protein chain. Specificity can now be explained in terms of the closeness of fit between enzyme and substrate at the active site. Highly specific enzymes require exact complementarity between enzyme and substrate surfaces, and even small changes in substrate will not be tolerated.

enzyme specificity

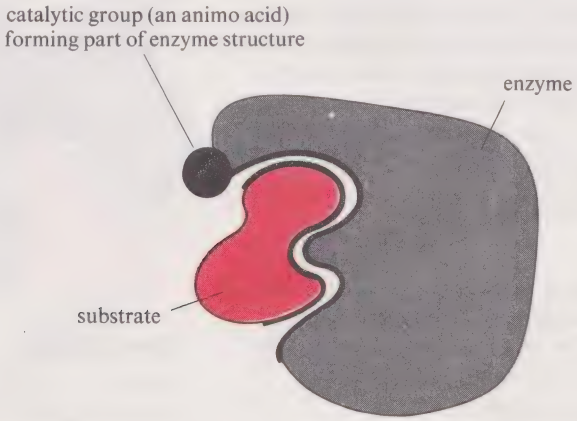


FIGURE 19 Catalytic group (black sphere) in enzyme active site, attacking substrate.

An example of such high specificity is given by an enzyme that catalyses the first reaction in glycolysis, one of the major energy-producing degradations of the cell, as you will discover in Unit 24. Its substrate, glucose, is selected from the vast

collection of other small molecules in the cell. No other molecule, not even another sugar, fits precisely enough to bind to the active site. The advantage of this particular high-specificity enzyme may be linked to the relative abundance of glucose compared with other sugars in the cell. If these rarer sugars are required for specialized roles, it would be disastrous for them to disappear in a degradative reaction. Furthermore, as you will see in Unit 24, cell chemistry consists of a series of interlocking enzyme-catalysed reactions. Incorrect choice of substrate by one enzyme would therefore have wide repercussions.

Very occasionally, it is advantageous to have a low-specificity rather than a high-specificity enzyme. Such a situation occurs in digestion*, where the animal is faced with a range of different macromolecules, all requiring to be hydrolysed to their component small molecules. A good example of a low-specificity digestive enzyme is pepsin, which can hydrolyse the peptide bond between a wide variety of amino acid pairs. The sidechain R groups (which may be chosen from a range of 20-odd varieties) are presumably not required to fit very tightly into the active site.

11.4 Classification of enzymes

Enzymes are most conveniently called by their *trivial* names. These are arrived at by adding the suffix *-ase* to either the substrate, or the reaction catalysed. (Noteworthy exceptions are those few like pepsin which retain the names they were given when first discovered, long before the days of systematic nomenclature.)

In 1956, the Commission on Enzymes was set up by the International Union of Biochemistry to rationalize the naming of enzymes. They described six categories of enzyme-catalysed reactions. The *hydrolases* for example, include the proteases that hydrolyse** proteins, the polysaccharases that hydrolyse polysaccharide, the nucleases that hydrolyse nucleic acids, the phosphatases that hydrolyse phosphate compounds, etc. The *transferases* are enzymes catalysing the transfer of small groups of atoms between molecules†, while the *oxidoreductases* catalyse oxidation or reduction reactions. An example of this last is lactic dehydrogenase, which catalyses the reaction $CH_3CHOHCOOH \xrightarrow[-2H]{} CH_3COCOOH$, which you will find in Unit 24. In addition to its trivial name an enzyme is given a *formal* name and a number by the International Union of Biochemistry. This describes in more detail the reaction catalysed, and further reduces the danger of ambiguities. For everyday use however, formal names are usually too cumbersome.

hydrolase, protease

11.5 Enzyme inhibitors

Since enzymes catalyse virtually every reaction in the cell, the question of how they are controlled has greatly occupied the minds of biochemists.

Like all catalysts, enzymes are very sensitive to small quantities of poisons or inhibitors. *Irreversible* inhibitors are compounds that react chemically with groups on the enzyme surface, becoming covalently bound to the enzyme and therefore not readily removed. These irreversible inhibitors are too potent to be any use as a biological control mechanism, and indeed, because of their irreversible action they may act as dangerous poisons. Examples are lead, mercury and cyanide, this last being an irreversible inhibitor of the final cytochrome (cytochrome *a*) in the mitochondrial electron transport chain, which will be described in Unit 24‡.

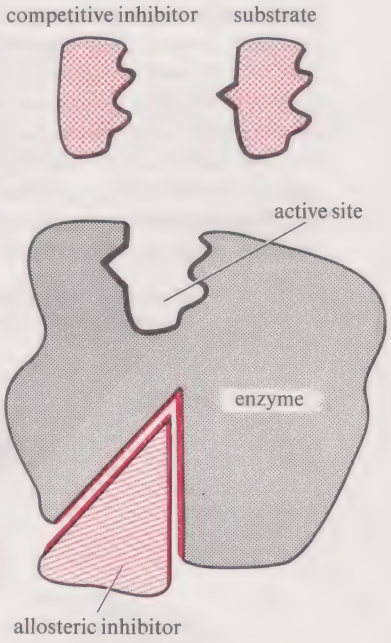


FIGURE 20 Binding of substrate, competitive inhibitor and allosteric inhibitor to enzyme surface. Thick black lines indicate surfaces that are complementary on enzyme and substrate—or enzyme and competitive inhibitor. Thick red lines indicate surfaces that are complementary on enzyme and allosteric inhibitor.

* Digestion is further described in Unit 24.

** *Hydrolysis* is a reaction in which an organic compound is split into two by reaction with water. The OH part of the water molecule attaches to one product, and the H atom attaches to the other. You have already come across this reaction in TV 16, which describes the formation of an ester by *condensation*—a reaction that is the reverse of hydrolysis:

$$RCO\dot{}OR' + H_2O \rightarrow RCOOH + R'OH$$

† The enzymes catalysing the transfer of amino groups between amino acids (which you will meet in Unit 24, Section 5.4.2) are known as transaminases, an abbreviation of the term amino group transferases.

‡ As you will see from Unit 24, without cytochrome *a*, all oxidation processes in the cell would grind to a halt—hence the great toxicity of cyanide to living organisms.

Reversible inhibition, where the inhibitor is not covalently linked to the enzyme, may be used by the organism as a means of control. A reversible inhibitor may closely resemble the true substrate and therefore compete with it for binding to the active site (see Figure 20). Such compounds are termed *competitive inhibitors* and their effect can be overcome simply by increasing the concentration of substrate.

The other major body of reversible inhibitors are the *allosteric inhibitors*. These have a structure very different from that of the substrate and therefore do not bind to the active site but to another area, the allosteric ('other shaped') site. Here they induce a *change in enzyme shape*, which is relayed across the enzyme from the allosteric to the active site (see Figure 21). A change in shape of the active site, as you might expect, has a radical effect on catalytic activity. The enzyme becomes less efficient at either binding substrate, or at aligning its catalytic groups, or both. Whatever the means, allosteric inhibitors cause a drop in the observed rate of enzyme-catalysed reaction. In contrast to competitive inhibitors, allosteric inhibitors have no need to resemble the substrate in any way. This type of inhibition therefore opens up all kinds of possibilities for control of enzyme activity. Molecules produced in one series of chemical transformations may inhibit an enzyme catalysing a quite unrelated reaction. Allosteric inhibition thus provides a means of communication between enzymes catalysing different series of chemical transformations in the cell. Its importance as a control mechanism cannot be overestimated*.

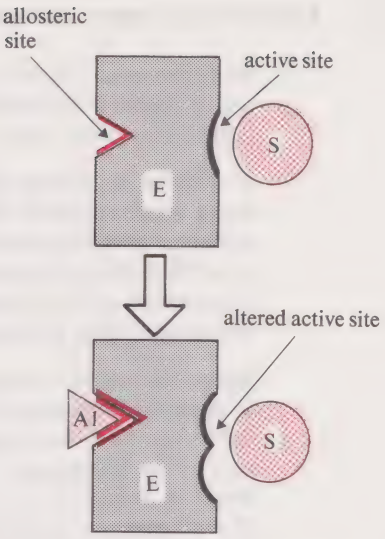


FIGURE 21 Allosteric inhibition of enzymic catalysis. Binding of allosteric inhibitor (AI) at its specific recognition site (outlined in red) on the surface of enzyme (E), relays a shape-change message to the active site (outlined by thick black line), such that substrate (S) no longer fits.

11.6 Methods for studying enzyme activity

Enzymes play a major part in the control of all chemical reactions in the body. Therefore it is not surprising that many experiments aimed at understanding basic biochemical concepts revolve around the measuring of enzyme activities. So too do an increasing number of the diagnostic tests performed in hospital pathology laboratories. It is therefore essential to have some idea of the kind of approach used in designing *enzyme assays*—methods for measuring enzyme activity.

enzyme assay

In any enzyme assay, what is measured is the *rate* at which substrate is converted to product. This is usually done by following some change in the reaction solution (for example, colour intensity) known to be related to substrate or product concentration. The *activity* of different enzymes, that is, *the amounts of substrate they convert to product in a given time, under optimum conditions*, can then be compared.

The solution in which the enzyme activity is to be measured is known as the *incubation medium*. Its makeup should be such that reaction takes place under optimum conditions. These vary for each enzyme. Temperature, pH, presence of inhibitors or activators can all influence the rate of reaction. So too can concentration of both enzyme and substrate, and all these factors must therefore be standardized.

incubation medium

How is the amount of substrate converted to product actually measured? The commonest methods use colorimeters or spectrophotometers, instruments that detect changes in the light-absorption properties of solutions as one molecule is converted to another. Sometimes however, an enzyme-catalysed reaction generates a proton (H^+) as one of its products. The reaction rate can then be followed by the rate at which alkali must be added to prevent the pH of the solution from falling. Still other methods of enzyme assay use radioactive substrates, and measure the rate at which the radioactivity is lost from substrate to product.

In the Home Experiment you will use a very simple method of observing the activity of the stomach enzyme, pepsin. This catalyses the conversion of insoluble protein (seen as a cloudy suspension in water) to its component amino acids and peptides (seen as a clear solution in water).

* Equally important in the cell is allosteric *activation*, where a similar mechanism is involved. Here, the shape change induced by the molecule binding to the active site causes the enzyme to *increase* (rather than decrease) its activity.

11.7 Home Experiment: the action of pepsin on egg white

Study comment In this Home Experiment we aim to teach further properties of proteins, particularly those of enzymes. The Experiment also illustrates some of the points described in earlier Sections of this Unit.

In addition to reading the text of this Section, you will need 2 hours for doing the Experiment. Writing up the results of your experimental work, and some questions relating to the design of the experiment, may form part of a TMA. Even if you decide not to do Part IV, the optional part of the Experiment, which concerns pH optima, you will still need to read the instructions there in order to fulfil the Section Objectives.

Before starting the Home Experiment you are advised to read carefully through the Introduction (Section 11.7.1), and to study Figure 22. This shows you how the substrate (egg white) is divided up between the four parts of the Experiment. The layout of the first three Parts (I, II and III) is shown in Table 8. As you may need to refer constantly to these, both Figure 22 and Table 8 are duplicated on a looseleaf sheet. The results for Parts I–III should be recorded on Table 10, also printed separately.

The layout for Part IV of the experiment is shown in Table 11, printed separately with Table 10. You should record your results for Part IV on this.

You should also read the instructions on the use of the gas burner, and the Safety Instructions relating to HCl and pepsin in the Home Experiment Kit booklet (Part 2) and keep them handy. You should also check Section 11.7.2 of this Unit to collect the items you are asked to provide.

Having performed this Home Experiment you should be able to:

1 Show how the pepsin clarification reaction may be used to demonstrate the following concepts: specificity of enzyme action, protein denaturation, pH optima.

2 Design simple controls for experiments involving enzyme-catalysed reactions.

3 Design simple experiments involving enzymes other than pepsin, given details of the nature of the reaction catalysed.

11.7.1 Introduction

This Home Experiment reproduces in the test-tube what happens to the white of a boiled egg after it disappears from view down your throat. The main protein component is ovalbumin, which we shall refer to as *egg-white protein*; also present are traces of lipid.

In the stomach, the protein in the egg is attacked by a low-specificity protease, pepsin. Hydrolysis of the peptide bonds between amino acids in the egg-white protein chain breaks the chain down to peptides, some few amino acids long. In this Home Experiment you will see the effect of pepsin on a fine suspension of egg-white particles. The suspension simulates the physical form of the egg white by the time it has been chewed around in the mouth and churned up in the stomach. In the experiment, hydrolysis by pepsin reduces the suspension to short water-soluble peptides, so that what you will see is the original cloudy suspension becoming clear. We shall refer to this phenomenon as *clarification*.

pepsin

clarification

It is important to emphasize that pepsin is an unusual enzyme. It operates under the very acid conditions of the stomach, where gastric juice secreted by the stomach wall contains $0.1\,\text{mol}\,l^{-1}$ HCl and has a pH of around 1. Most other enzymes would be denatured at such a low pH.

Your egg-white protein may contain traces of that other egg-white component, lipid. This of course will not be attacked by protease, and you may be able to see it as a very faint cloudiness persisting even after the egg-white protein has dissolved.

Home Experiment

The experiment falls into four parts. Part I concerns the preparation of egg-white suspension, and includes a simple demonstration of the clarification effect of pepsin. Part II of the experiment is a repeat of Part I, but with appropriate controls. Here you need to prove that it is indeed the enzyme, and nothing else that is clarifying the egg-white suspension. Part III (which can be done simultaneously with Part II) uses the experimental design set up in Part I to demonstrate two of the fundamental properties of enzyme-catalysed reactions. Part IV is optional; it demonstrates the effect of one variable, pH, on the rate of the enzyme-catalysed reactions.

Since Part IV starts off with substrate in water and not—as in Parts I, II, and III—in acid, you will be told to set aside for it 11.5 cm^3 of egg-white suspension while you are doing Part I.

Now look briefly at Figure 22, which summarizes the layout of the whole experiment. You will find it useful to refer back frequently to this central figure, and also to Table 8. These are duplicated on a looseleaf sheet so that you can have them in front of you while you are working.

11.7.2 Experimental details

Apparatus needed from the Home Experiment Kit

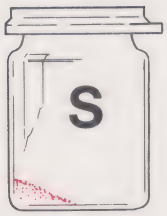
Black card (in Part 1 of the Kit) or any piece of dark material will do
Stop-watch
Gas burner
Tripod
Gauze
Test-tube holder
Test-tube rack
17 test-tubes
Felt-tipped pen
2 × 1 cm^3 syringes with long needles
3 × 2 cm^3 syringes with long needles
1 × 20 cm^3 syringe
1 × 250 cm^3 beaker
1 × 500 cm^3 beaker
1 × 100 cm^3 quickfit conical flask
1 × 25 cm^3 measuring cylinder
1 × 100 cm^3 measuring cylinder
2 dropping pipettes
1 pipette bulb (1 cm^3)
Squeeze bottle for distilled water

Chemicals needed from the Home Experiment Kit

0.25 $mol\,l^{-1}$ citric acid
8.9% HCl
Pepsin
Egg-white protein (ovalbumin)

Items you are asked to provide

One mug, glass or jam pot in which you can stand a test-tube
1 raw egg (optional)
Clock or wristwatch (second hand not required)
Distilled water (50 cm^3)
Matches for lighting gas burner
Gas cylinder



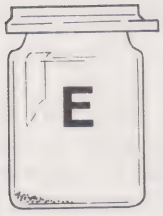


FIGURE 22 Plan of pepsin experiment.

200 mg substrate. Redissolve in 20 cm^3 H_2O; boil; cool.

40 mg enzyme. Redissolve in 20 cm^3 H_2O.

PARTS I–III

Take	8.5 cm^3	egg-white suspension
Add	0.5 cm^3	8.9% HCl
	11.0 cm^3	H_2O
Total	20.0 cm^3	acidified substrate

Take 2 cm^3 for each of the following:

I — standard assay

control—no enzyme

II — control—boiled enzyme

III — standard assay with extra substrate

standard assay, using boiling, concentrated HCl instead of enzyme

PART IV

Take	11.5 cm^3	egg-white suspension
Add	10.5 cm^3	H_2O
Total	22.0 cm^3	non-acidified substrate

Take 2 cm^3 for each of the following

IV

		pH
P	P_c	0.5
Q	Q_c	1.1
R	R_c	1.8
S	S_c	2.2
T	T_c	6.0

TABLE 8 Layout for Parts I, II and III of the Home Experiment.

Tube No.	Acidified substrate	Pepsin	HCl or water	Reaction conditions
A, B Part I	2 cm^3	1 cm^3	0	standard assay
	2 cm^3	0	1 cm^3 H_2O	control (no enzyme)
C	0	0	3 cm^3 H_2O	water only
D Part II	2 cm^3	1 cm^3, boiled	0	control (boiled enzyme)
A′ Part III	further 2 cm^3 added to tube A	as A; no further additions	0	further substrate added to tube A
E	2 cm^3	0	1 cm^3 8.9% HCl	boiling, strong acid

PART I

Aim

To prepare the egg-white suspension, and demonstrate its clarification by pepsin.

Method

1 *Preparation of egg-white suspension* (This is one of the most important parts of the experiment. Because pepsin activity depends very much on the physical state of the egg-white, you should follow these instructions as closely as possible)

Like many proteases, pepsin works best on denatured proteins. The simplest way to denature egg-white protein is to boil it. The only difference between boiling an egg as you would normally to cook it and preparing the egg-white suspension is that here you are aiming at the preparation of minute, finely divided egg-white particles, whereas in cooking you would be satisfied with the egg white in a solid lump. Because pepsin is so influenced by the physical state of the egg-white suspension (i.e. the size of the suspended particles)* we have tried to standardize the procedure by providing you with a freeze-dried extract of egg-white protein, rather than letting you start with your own raw egg. If you want to try starting from whole egg, see Appendix 2.

You are provided with 200 mg of freeze-dried egg-white protein in a small bottle. (Keep this tightly closed until you are ready to use it. Damp protein powders do not store well.) The aim is first to suspend the protein in 20 cm^3 of water, as a finely divided powder, and then to denature it by heat.

(a) Fill the 25 cm^3 measuring cylinder up to the 20 cm^3 mark** with distilled water. Remove the cap from the egg-white bottle, taking care not to lose any of the powder. Pour in 10–12 cm^3 of water from the measuring cylinder. Put the cap on again tightly. Invert the bottle sharply, but taking care not to cause too much frothing. Continue inverting for 3 minutes. The powder will first come away from the bottom, and then disperse as an even suspension. A few smallish lumps will not matter. Transfer this suspension to a 100 cm^3 conical flask. Pour the rest of the water left in the measuring cylinder into the bottle to rinse it, and add this rinsing to the bulk of the egg-white suspension in the conical flask.

You are now ready to denature your egg-white suspension by heating it.

(b) Light the gas burner, setting it on a medium flame with air hole open. Attach the test-tube holder to the neck of the conical flask, and use this to hold the flask at an angle in the flame (Figure 23), *shaking gently* to make sure the suspension is evenly heated. (Remember to point the flask away from you and anybody else.) Just before the suspension begins to boil, it should become much cloudier, so that it now looks like milk.

STOP heating at this point, and immediately COOL the suspension by holding the conical flask at an angle under a cold water tap (Figure 24) for at least 3 minutes.

You should now have the desired suspension of egg-white, with the protein denatured and the particles finely divided.

(c) Using a 1 cm^3 syringe, transfer as accurately as possible, 0.5 cm^3 of 8.9% HCl to the 25 cm^3 measuring cylinder†. (CARE: 8.9% HCl is corrosive.) Add egg-white suspension up to the 9 cm^3 mark, using a dropping pipette and bulb for the last few cm^3. Then make up to the 20 cm^3 mark by adding distilled water. Stir to mix.

* This dependence of pepsin activity on particle size may explain the old wives' tale that 'hard-boiled eggs are indigestible'. There are no small particles.

** Up to the mark means that the meniscus is on the 20 cm^3 mark. See the Home Experiment Notes for Unit 15 if you are unsure about this.

† Use this syringe only for acid unless you are able to wash it out extremely well. You will need it again in Part III.

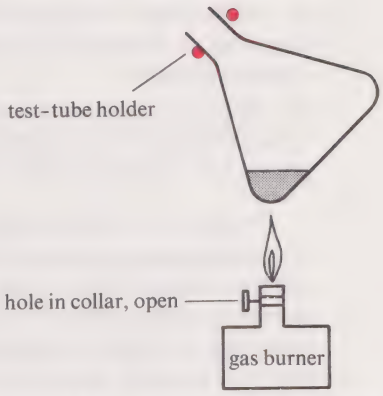


FIGURE 23 Denaturating the egg-white suspension.

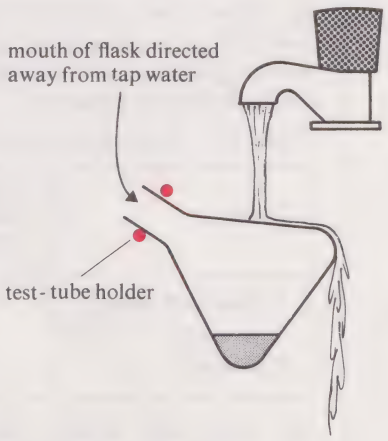


FIGURE 24 Cooling the egg-white suspension.

CAUTION

Now stand the measuring cylinder in a large beaker (500 cm^3) to prevent it being knocked over. Label the measuring cylinder 'acidified egg white'. You can keep this suspension at room temperature, unless you plan to store it for more than twenty-four hours. If you do need to store it overnight, it is best kept cool, for example, in ice-water in a thermos flask, or (still in its anti-spill beaker) in a refrigerator.

The rest of the egg-white suspension in the conical flask should be stored, if possible in a refrigerator until you are ready to do Part IV.

2 *Preparation of pepsin solution*

You are provided with another air-tight bottle containing 40 mg of pepsin as a freeze-dried powder. Again, keep the bottle tightly closed until you are ready to use it. The pepsin is to be dissolved in water to give a final concentration of 2 mg cm^{-3}. This is stable for at least two days at room temperature, and longer if kept at 5 °C. If you do keep it at room temperature, place it away from obvious heat sources like the gas burner, radiators, or sunny windows. Remember that it may dissolve protein, for example your skin, if you spill it.

(a) Place 20 cm^3 of distilled water in the 100 cm^3 measuring cylinder, measuring as accurately as possible, and pour this slowly into the bottle containing pepsin.

(b) Replace cap. Invert the bottle about three times to ensure thorough mixing (but avoid excess frothing). You should now have a clear solution of pepsin, and are ready to start the reaction.

3 *Procedure for demonstrating clarification reaction*

(a) Look carefully at Table 9. Tube A is to be the one in which the enzyme-catalysed reaction takes place. Tube B is the control, containing water instead of enzyme. Tube C (which contains just water) is for comparison, to show what the egg-white preparation will look like after clarification. Note that the *standard assay set-up* is as follows: 2 cm^3 substrate + 1 cm^3 enzyme.

(b) Label three test-tubes, A, B and C.

(c) Place 2 cm^3 of acidified egg white into each of tubes A and B, using a 2 cm^3 syringe; with another 2 cm^3 syringe, place 1 cm^3 of distilled water into B and approximately 3 cm^3 into C (as shown in Table 9). Shake tube B briefly to mix the contents.

TABLE 9 Standard procedure for demonstrating pepsin activity

Tube	cm^3 of acidified egg white	cm^3 of pepsin solution	cm^3 of water
A (standard assay)	2	1	0
B (control)	2	0	1
C (water)	0	0	3

(d) Fill a 1 cm^3 syringe with pepsin solution. Make sure you keep this syringe for dispensing only this pepsin solution. Start the stop-watch and immediately transfer the pepsin solution sharply from the syringe into tube A. Shake briefly to ensure thorough mixing.

(e) Compare tube A with tubes B and C after 5, 10 and 30 minutes, using the black card as a dark background against which to hold the tubes. Note whether the contents of the tube are clear (like the water in tube C) or still cloudy. Record your observations in Table 10 (p. 48) by writing 'R' (for cleaR) or 'Y' (for cloudY) in the appropriate space. Two copies are provided as looseleaf sheets, one for your use while doing the experiment, and an extra one for you to send to your tutor if necessary. (If your suspension starts to clear rapidly, and then remains obstinately the same for several hours, you may be demonstrating the ineffectiveness of pepsin towards lipid. This observation would also emphasize the need for control tubes—only by comparison with Tube B is the initial clarification convincing.) Keep Tube A on one side in the test-tube rack for use in Part III.

PART II (This can be done simultaneously with Part III)

Aim

To repeat Part I using a second control—boiled enzyme.

Introduction

Although you have now (we hope!) shown how pepsin can clarify a cloudy egg-white suspension, and have demonstrated from one control (tube B in the previous Part) that without enzyme solution there is no clarification, you have by no means proved that it is the protein pepsin in this solution that catalyses the reaction. It could be a non-protein molecule also present in the pepsin solution.

The most direct way of proving the participation of protein is first to *denature* it by boiling, and then see whether it still catalyses the clarification reaction. Using heat-denatured enzyme in this way is an important routine control in enzyme investigations. In the control tube (see D below), conditions must be identical with those in the standard assay, except for the presence of boiled enzyme.

Method

1 *Preparation of boiled pepsin* Using a $1\,cm^3$ syringe, place $1\,cm^3$ of pepsin solution into a tube labelled D. Then place the tube in a $250\,cm^3$ beaker of boiling tap water, and leave it there for at least five minutes. Remove the tube, and cool it by standing in a mug or jar of cold water for at least a further three minutes.

2 You are now ready to start the reaction, by adding egg-white suspension. (Note that the contents of D are identical to those in tube A of the previous experiment, but contain boiled enzyme.)

3 Add $2\,cm^3$ of acidified egg-white suspension to tube D, shake briefly to mix the contents and start the stop-watch. (Alternatively, for the longer time intervals, you may find it easier simply to record the time on your watch.)

4 Record your observations in Table 10.

PART III (This can be done simultaneously with Part II)

Aim

To demonstrate some of the key characteristics of enzyme-catalysed reactions.

Introduction

The pepsin-catalysed clarification reaction can be used to demonstrate two of the points we mentioned in Section 11 of this Unit.

1 Like all true catalysts, the enzyme is not used up during the catalytic reaction, whereas the substrate is. To demonstrate this you have simply to add more substrate (egg-white suspension) to a standard assay tube, such as tube A which you used in Part I.

2 Because of their effect on the activation energy of a reaction, enzymes are able to catalyse, under very mild conditions, reactions that would otherwise require extreme conditions of temperature and pH, etc. You can demonstrate this by carrying out the clarification reaction *without* pepsin. The suspension still clears, but needs strong acid (8.9% HCl), high temperature (boiling water) and, as you will see in tube E below, very much longer incubation times.

Method

1 Take tube A (from Part I) which already contains the clarified suspension. Repeat the clarification reaction by adding a further $2\,cm^3$ of egg-white suspension to the *same* tube. Relabel this tube A′. (The suspension

TABLE 10 Results of Parts I–III of pepsin Home Experiment

	Tube	Appearance of suspension after various time intervals*					Space for recording any further observations
Part I		5 min	10 min	30 min	60 min	min**	
	A standard assay				—	—	
	B no enzyme				—	—	
	C water only				—	—	
Part II	D as A, but boiled enzyme	—	—				
Part III	A′ more egg white added to tube A				—	—	
	E no enzyme; strong boiling acid	—	—				

* Write 'R' (for cleaR) or 'Y' (for cloudY).
** Further time interval (specify your own).

should clarify again, even though no further enzyme has been added. It may take longer than before, but this is because the solution is now more dilute, not because the enzyme is exhausted.)

2 Into a tube labelled E, place 2 cm^3 of acidified egg white and 1 cm^3 of 8.9% HCl. Place the tube in the boiling-water bath. Note the time. Observe the tube after 30 min and 60 min.

3 Record your observations for tubes A′ and E in Table 10.

PART IV ★

Aim

To find the optimum pH for the pepsin-catalysed clarification of egg white.

Introduction

In this experiment you are asked to compare the activity of pepsin at five different pHs. It will not be possible to plot as precise a curve as in Figure 17 because you will have too few points, but you should be able to get some idea of the optimum pH for pepsin activity towards egg white.

As each incubation medium must have a control, you will have to dilute the egg-white suspension to a concentration slightly below that used in previous Parts of the experiment.

Method

1 Using the 20 cm^3 syringe, add 10.5 cm^3 of distilled water from a measuring cylinder to the non-acidified egg white left in the conical flask from Part I, step 1(c). This is the *substrate* for Part IV.

2 Preparation of 0.25 mol l^{-1} HCl. Using the same 1 cm^3 syringe as before, transfer 2 cm^3 of 8.9% HCl* (CARE 8.9% HCl is corrosive) into the 100 cm^3 measuring cylinder. Add distilled water up to the 20 cm^3 mark. Label this container 0.25 mol l^{-1} HCl.

CAUTION

* 8.9% HCl is, in fact, 2.5 mol l^{-1}.

TABLE 11 Layout and results of Part IV of pepsin Home Experiment.

Tube No.	pH	Volume of non-acidified substrate	Volume of acid	Volume of Water	Appearance after various time intervals		
					10 min	30 min	—
P	0.5	2 cm^3	1 cm^3 8.9% HCl	1 cm^3			
P_c	0.5	2 cm^3	1 cm^3 8.9% HCl	3 cm^3			
Q	1.1	2 cm^3	1 cm^3 0.25 $mol\,l^{-1}$ HCl	1 cm^3			
Q_c	1.1	2 cm^3	1 cm^3 0.25 $mol\,l^{-1}$ HCl	3 cm^3			
R	1.8	2 cm^3	0.3 cm^3 0.25 $mol\,l^{-1}$ HCl	1.7 cm^3			
R_c	1.8	2 cm^3	0.3 cm^3 0.25 $mol\,l^{-1}$ HCl	3.7 cm^3			
S	2.2	2 cm^3	2 cm^3 0.2 $mol\,l^{-1}$ citric acid	0			
S_c	2.2	2 cm^3	2 cm^3 0.2 $mol\,l^{-1}$ citric acid	2			
T	6.0	2 cm^3	0	2 cm^3			
T_c	6.0	2 cm^3	0	4 cm^3			

3 Arrange 10 tubes in the test-tube rack, labelled as follows: P, P_c; Q, Q_c; R, R_c; S, S_c; T, T_c. Transfer to each, 2 cm^3 of substrate and the appropriate volumes of acid or water as shown in Table 11. For the substrate, you can use the same 2 cm^3 syringe as for non-acidified egg-white suspension in Parts I–III. For water use the 1 cm^3 syringe you used before, and for acid use the clean 2 cm^3 syringe. Do *not* use the syringe in which you have been pipetting 8.9% HCl unless you rinse it out *very* thoroughly.

Note that the contents of the control tubes (P_c, Q_c, R_c, S_c and T_c) are identical to those of the experimental tubes, except that they contain extra water. This is to compensate for the pepsin solution that will be added to the experimental tubes in Step 5. The final incubation medium will comprise 2 cm^3 substrate, 2 cm^3 enzyme or H_2O, 2 cm^3 acid at correct pH (made by mixing H_2O, HCl or citric acid in appropriate proportions).

4 Shake each tube briefly to ensure thorough mixing of contents. The pH of each will now be as shown in Table 11, column 2. You are now ready to start the reaction.

5 Add 2 cm^3 of pepsin to each of tubes P, Q, R, S and T. Shake briefly to mix the contents.

6 After 10 minutes, observe each tube as before. Record your observations on one of the looseleaf copies of Table 11 and state what conclusions can be drawn about the effect of pH on pepsin activity. ★★

11.7.3 Writing up your Home Experiment

You should write up your Home Experiment in four sections: Aim, Method, Result and Conclusion. You might also like to refer to *HED**, Section 2.14. **HED**

Aim

This should be very short and should describe what you aimed to achieve. We suggest you simply use the titles given in Section 11.7.2 for each of Parts I–IV.

* The Open University (1979) S101 *The Handling of Experimental Data* (*HED*), The Open University Press.

Method

This section should describe the apparatus used, and the procedure followed, in sufficient detail to enable another person to repeat the identical experiment. However, there is no point in reproducing the notes given here, so we suggest you say something like: 'The experiment was performed as in Section 11.7.2, pp. 45–48, with the following modifications' (if you changed the method at all).

Result

This section should describe what actually happened without, at this stage, suggesting any reasons why. Results are often best described by a table or graph, and here we suggest you simply say something like: 'The results are shown in Table 9'. And then add any other observations you made that might be relevant, e.g. whether the suspension went absolutely clear, or whether a faint cloudiness persisted and, if so, for how long. For Part IV you might plot a graph similar to Figure 17, of pH against clarification time.

Conclusion

This should tie in with the aim of the experiment. In this section you may bring in background theory to try to explain your results, or to relate them to other known observations.

11.8 Objectives of Section 11

Now that you have studied Section 11 (including the Home Experiment on pepsin) you should be able to:

(a) Explain the specificity of an enzyme-catalyzed reaction, and the effects of temperature and pH on reaction rate, in terms of the molecular structure of the enzyme.

(b) Describe two types of enzyme inhibition, relating each to its role in the control of cell chemistry.

(c) Describe what you would expect to see when pepsin is incubated with egg-white suspension under various specified conditions.

(d) Explain the need for controls in experimental work, and be able to devise simple controls for an experiment involving enzymes.

To test your achievement of these Objectives, try SAQs 16–22.

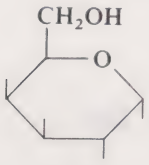
SAQ 16 (*Objective* (*a*)) If an enzyme is heat-stable for at least 30 minutes at 50 °C, has an optimum pH of 7, and can tolerate pHs within the range 4–8, under which of the following circumstances would you expect maximum enzymic activity, during a ten-minute assay?

(i) 50 °C and pH 7.5

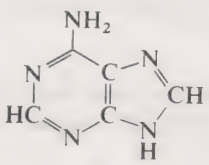
(ii) 70 °C and pH 7

(iii) 50 °C and pH 3.9

SAQ 17 (*Objective* (*b*)) Both of the following compounds (a) and (b) have been found to inhibit the activity of X, an enzyme whose natural substrate



(a)



(b)

is glucose. Which compound is most likely to be a competitive inhibitor and which an allosteric inhibitor of the enzyme? Give reasons for your answer.

SAQ 18 (*Objective* (*c*)) What differences would you expect in the rate at which pepsin clarifies an egg-white suspension, if the assay conditions described for the standard assay tube are modified as follows?

(i) Pepsin concentration is doubled.

(ii) A quantity of boiled pepsin equal to that of the unboiled pepsin already present is added.

(iii) An allosteric inhibitor of pepsin is added.

(iv) A pseudo-peptide is added, which binds to the same enzyme site as the peptide bond being hydrolysed in egg white.

(v) The pH is raised to 7.

For each question, select your answer from the following key, giving reasons for your choice.

KEY

A Clarification rate remains the same

B Clarification rate increases

C Clarification rate decreases

SAQ 19 (*Objectives* (*a*) *and* (*c*)) Would you expect pepsin to clarify a suspension prepared not from egg white but from (a) meat particles (b) olive-oil droplets? Give reasons for your answer.

SAQ 20 (*Objective* (*d*)) Enzyme X catalyses the reaction:

$$\underset{\text{compound S}}{\text{RCHOHCOOH}} \longrightarrow \underset{\text{compound P}}{\text{RCOCOOH}} + 2\text{H}$$

This reaction is measured by the increase in absorbance of light at 340 nm of Y, a dye which is added to the solution to 'collect' the H atoms liberated during conversion of S to P. When Y is reduced (that is, when it has combined with H atoms) its spectrum changes, giving a greater absorbance at 340 nm. This can be measured with a spectrophotometer.

Select from A–J in the following key those solutions you would mix together in order to measure the activity of enzyme X in blood.

KEY for SAQs 20–22

A Blood

B Compound S

C Compound P

D Buffer

E Dye Y

F Competitive inhibitor of X

G Allosteric inhibitor of X

H Water

I Strong acid

J Lead solution

SAQ 21 (*Objective* (*d*)) It is suggested that traces of lead in drinking water may be toxic, because they inhibit enzyme X. Which of the solutions A–J listed in the key would you mix together, and what volumes of each would you take, in order to test this hypothesis? *Note* *Two* assay tubes will be needed, one to act as control. (Tables 9 and 11 in Section 11.7 may be useful here.)

SAQ 22 (*Objective* (*d*)) The water in SAQ 21 is replaced by 1 cm^3 of an unknown solution. The absorbance at 340 nm then remains constant, even in the presence of enzyme. Which of the solutions A–J in the key is most likely to cause this effect? (More than one may be chosen.)

Objectives

Now that you have studied Unit 23, you should be able to:

1 Define correctly, recognize the best definitions of, and distinguish between true and false statements concerning the terms, concepts and principles listed in Table A. (SAQ 11)

2 Identify endoplasmic reticulum, mitochondria, chloroplasts, the cell membrane and the nuclear membrane in photographs. (SAQ 1)

3 Estimate the size of cellular structure in photographs, given a scale or magnification. (SAQ 2)

4 Choose an appropriate method of centrifugation, given the relative sizes and densities of organelles in a homogenate. (SAQ 3)

5 Number the carbon atoms in both straight-chain and ring formulae of glucose. (SAQ 4)

6 Reproduce the ring formulae of α and β-glucose, using the convention depicted in Figures 4d and 4e. (SAQ 4)

7 Given the structures of different sugars, point out whether they vary in the lengths of their carbon chains, in the relative positions of H, OH pairs on ring carbons, or in the size of their rings. (SAQ 6)

8 Recognize from their formulae, the following compounds: long-chain fatty acid, neutral fat. (SAQ 7)

9 Show by means of a simple equation, how neutral fats are formed from fatty acids. (SAQ 7)

10 Recognize from formulae and equations the following reactions:

(a) esterification of glycerol by fatty acids

(b) oxidation (or reduction) by removal (or addition) of H atoms to the carbonyl group in carboxylic acids

(c) reduction by addition of H atoms across a double bond in carboxylic acids

(d) reaction between phosphate and carboxylic acid groups (SAQ 7)

11 Reproduce the general formula for an amino acid, and the specific formulae for glycine and alanine. (SAQ 8)

12 Given the formula of an amino acid, state whether it is small or large and bulky, negatively or positively charged, and whether or not it contains reactive SH or OH groups. (SAQ 8)

13 Recognize the general formulae of purine and pyrimidine bases, and show diagrammatically how they are linked together with phosphate and sugar to form a nucleotide. (SAQ 9)

14 List the four main classes of compound found in the cell, the elements they contain and the small organic molecules of which they are built up. (SAQs 10 and 11)

15 Distinguish the following molecules from their formulae: monosaccharide (i.e. sugar), fatty acid, glycerol, amino acid, nucleotide. (SAQ 12)

16 Explain what is meant by primary structure in polysaccharides, proteins and nucleic acids. (SAQs 13 and 14)

17 Describe in general terms (without formulae) the primary structures of support and food-storage polysaccharides, and support and catalytic proteins. (SAQ 14)

18 Explain how the biological role of a globular protein is influenced by both its primary and its higher-order structure. (SAQs 13–15)

19 Explain in terms of weak bonding and higher-order structure, why proteins lose their biological activity at high temperatures and extremes of pH. (SAQs 13 and 15)

20 Explain the specificity of an enzyme-catalysed reaction, and the effects of temperature and pH on reaction rate, in terms of the molecular structure of the enzyme. (SAQs 16 and 19)

21 Describe two types of enzyme inhibition, relating each to its role in the control of cell chemistry. (SAQ 17)

22 Describe what you would expect to see when pepsin is incubated with egg-white suspension under various specified conditions. (SAQs 18 and 19)

23 Explain the need for controls in experimental work, and be able to devise simple controls for an experiment involving enzymes. (SAQs 20–22)

Appendices

Appendix 1 Physical principles of centrifugation

For a very small particle such as a subcellular organelle, the settling velocity is given by the equation

$$v = \frac{2r^2g}{9\eta}(\rho_p - \rho_w) \qquad (1)$$

in which r stands for the radius of the particle, assumed to be spherical, g the acceleration due to gravity, ρ_p the density of the particle, ρ_w the density of water, and η the viscosity of water. If the particles are not spherical, the numerical factor is different, but the velocity still depends on g, on the density difference $(\rho_p - \rho_w)$ and, above all, on the size of the particle, i.e. on r.

To get an idea of the settling velocity of a very small particle, you might like to calculate v from the following data: particle radius $r = 0.001\,\text{mm}$; particle density $\rho_p = 1.100 \times 10^3\,\text{kg m}^{-3}$; density of water $\rho_w = 1.00 \times 10^3\,\text{kg m}^{-3}$, viscosity of water $= 1.00 \times 10^{-3}\,\text{N s m}^{-2}$; $g = 9.8\,\text{m s}^{-2}$.

We found $v = 2.2 \times 10^{-7}\,\text{m s}^{-1}$, which is about 0.8 mm per hour. Subcellular organelles can be much smaller than 0.001 mm in 'radius'. So separation of organelles by sedimentation alone could be an impossibly slow process.

You can see from equation 1 that this process can be speeded up (that is, v will increase) if g is increased. This can be achieved by centrifugation, in which the homogenate is rotated at very high speeds.

A large, inwardly directed (centripetal) force would be needed to keep the particles moving along a circular path at a constant distance from the axis of rotation, that is, to keep them at a fixed position inside the container. Since no such centripetal force can act on the particles while they are still in suspension, they cannot stay where they are, and they move away from the axis of rotation, towards the bottom of the container. Once they reach the bottom, the container itself can provide the centripetal force needed to keep them moving along their circular path around the axis. Thus the particles in the container behave as if there were a radially outward force on them (towards the bottom of the container). This force is called *centrifugal force*. It is greater if the speed of rotation is greater, or if the radius of the circle is smaller. Thus the effect on the particles in the container of rotating it in this way is the same as that of allowing them to settle under the act of a greatly increased gravitational force. The settling process will be greatly speeded up.

Under these conditions, even the smallest subcellular organelles of the homogenate can be made to fall towards the bottom of the container in a reasonable time (so long as their density is greater than that of the surrounding solution), and they can thus be isolated separately from a cell homogenate.

Appendix 2 How to prepare an egg-white suspension suitable for demonstrating pepsin activity, starting from a raw egg

Method

(a) Make yourself a *wide-tipped* pipette, by breaking about 1 inch off the end of one of the dropping pipettes provided in the Home Experiment Kit. (Wrap the end in a piece of tissue paper first, to avoid cutting yourself.) Attach a 1 cm^3 bulb to this pipette.

(b) Crack an uncooked egg so that at least half the white can be separated from the yoke and poured into a cup.

(c) Place 10 cm^3 of tap water into a test-tube.

(d) Transfer four drops of egg white into the test-tube, using the wide-tipped pipette. (Practise this first, by simply drawing egg white into the pipette, and releasing it back into the cup. Aim at getting a clearly suspended drop that will fall from the tip of the pipette under its own weight.) When pipetting into the solution in the tube, it is important to stir well* *after adding each drop.*

(e) Hold the tube, by means of the test-tube holder, in a medium flame (collar open). Keep the suspension of egg white well shaken. Just before boiling point the suspension should thicken and start to look like milk. Stop heating at this point, and cool as in Part I, step 1(b) of the pepsin experiment (Section 11.7). You now have an egg-white suspension comparable with that in Part I, step 1(b).

You can treat it in exactly the same way as the suspension prepared from freeze-dried egg-white powder, following instructions from Part I, step 1(c) onwards. However, the particle size of your home-made egg-white suspension may be less easy to standardize than ours, and since this is an important factor in determining pepsin activity, you must be prepared for rather different clarification times. For a given suspension, of course, times should be reproducible.

Recommended reading for Units 23–25

Rose, Steven (1979) *The Chemistry of Life*, revised edn, Penguin.

Yudkin, M. and Offord, R. E. (1971) *A Guidebook to Biochemistry*, 3rd edn, Cambridge University Press. (New edition in preparation.)

Acknowledgements

Grateful acknowledgement is made to the following sources for illustrations used in this Unit:

Figure 11 (*a*) *and* (*c*) from R. E. Dickerson and I. Geis (1969) *The Structure and Action of Proteins*, W. A. Benjamin Inc.; *Figure 12* (*c*) courtesy of Professor J. D. Dodge.

* You will need to find something to stir with. A glass rod may go through the bottom of the tube if you are not careful. A plastic toothbrush handle or similar object might be better.

List of Filmstrips for Unit 23

Filmstrip 23.1,	Frame 1	*Tradescantia* plant with flowers.
	Frame 2	Flowers of *Tradescantia.*
	Frame 3	Stamens and hairs in a *Tradescantia* flower.
	Frame 4	Part of two hairs from a *Tradescantia* flower.
	Frame 5	One cell from a hair from a *Tradescantia* flower.
	Frame 6	A cell from the lining of human cheek.
	Frame 7	Human red blood cells.
Filmstrip 23.2,	Frame 8	Two types of cell from the wood of a plant stem.
	Frame 9	Cells from the upper epidermis of a leaf.
	Frame 10	Epidermis cell and cells from the palisade layer of a leaf.
	Frame 11	Part of a section across a leaf.
	Frame 12	Surface view of cells of the lower epidermis of a leaf.
Filmstrip 23.3,	Frame 13	A fibrous protein.
	Frame 14	A globular protein, lysozyme.
	Frame 15	An enzyme, lactic dehydrogenase.

SAQ answers and comments

SAQ 1 (a) a Endoplasmic reticulum. b Mitochondrion. c Outer membrane of nuclear envelope. d Nucleus. e Ribosomes. f Mitochondrion. g Chloroplast.

(b) It is eukaryotic, because it contains membrane-bounded organelles.

(c) It is a plant cell, because it contains a chloroplast.

SAQ 2 Mitochondrion. The width is approximately 0.7 μm. The magnification of the image is 30 000 times life size. So at this scale 1 μm would be 30 mm. The average width of the mitochondrion is approximately 20 mm, so its real width is 20/30 μm = 0.7 μm.

SAQ 3 (The bracketed sections of this answer are optional information; they derive from the discussion of physical principles of centrifugation given in Appendix 1.) *Differential centrifugation* should be used to separate the organelles from homogenate A. [The ten-fold differences in size will mean hundred-fold differences in settling rate, if the densities are nearly the same. So the organelles *a*, *b*, *c* will be easily separable, *a* settling well before *b* and *b* well before *c*.] *Density-gradient* centrifugation should be used to separate homogenate B. Although the organelles have very nearly the same size, they *could* be separated by differential centrifugation, as their different densities would mean different settling rates. But the density differences are not large, so the separation would be rather poor. [But if a three-layer fluid were used, with densities of 1 280, 1 170 and 1 050 kg m^3 (bottom to top layer, respectively), the separation of *x*, *y* and *z* between the three layers would be complete.]

SAQ 4 (i) True. See Figure 4 for numbering of carbon atoms.

(ii) False. The phosphorylated sugar is galactose-6-phosphate.

SAQ 5 (i) B and C. Both are 6-carbon sugars.

(ii) C. Sugars differ at C-4.

(iii) A, B C. The first sugar is 6-carbon (glucose), and the second is 5-carbon (ribose); the first has a *6-membered* and the second has a *five-membered* ring.

SAQ 6 The completed paragraph should read: 'By bicycling rather than driving to work, an obese person may use up reserves of *neutral fat*. These will have been deposited by reaction between long-chain *fatty acids* and *glycerol*. Of these various compounds, those that are insoluble in water may be described as *lipids*.' (In fact, only glycerol is water-soluble, and therefore cannot strictly be called a lipid.)

SAQ 7 (i) A. The starting compound may be written RCOOH but has too long an R group to be a simple carboxylic acid. (Note that the product could also have been written $CH_3(CH_2)_7CH_2-CH_2(CH_2)_7COOH$.) (ii) C. Note how two different types of fatty acid are found in this particular neutral fat.

SAQ 8 (See Table 5.) (i) False. Tryptophan is large and bulky, and has no positive charge. (Lysine and arginine are the only two amino acids with positively charged sidechains.)

(ii) True.

(iii) True. (Methionine has S, but not in the form of an SH group.)

(iv) True.

SAQ 9 D. In A, the basic component is an amino acid, not a purine or pyrimidine; in B and C the sugar is 6-C glucose, not 5-C ribose or deoxyribose. Furthermore, the phosphate in B is not at the end.

SAQ 10 All four classes of compound contain C, H, O. Only proteins and nucleotides contain N, and only nucleotides contain P. Iron, manganese and sulphur are found only in certain proteins.

SAQ 11 All the proteins would produce amino acid; the fats would produce fatty acid and glycerol; both starch and cellulose would produce a sugar (glucose). (The reason why lettuce is a good slimming food, whereas bread is not, is that animals lack the equipment (enzymes) necessary for breaking down lettuce carbohydrates (cellulose) in the gut.)

SAQ 12 (i) Sugar. This has no COOH (acid group), and has the general formula $C_nH_{2n}O_n$ characteristic of sugars.

(ii) Fatty acid. This has an acidic group (COOH) but no nitrogen and fits the general formula RCOOH.

(iii) Amino acid. This contains both NH_2 and COOH groups and fits the general formula $RCH(NH_2)COOH$.

SAQ 13 True. (See Section 10.4).

SAQ 14 (i) False. Primary structure concerns both the nature of component small molecules, and the *way in which they are strung together* (for example, cellulose and starch are both glucose polymers, but are linked in different ways).

(ii) False. This statement would be true for *support* polysaccharides.

(iii) True.

(iv) False. This statement would be true for *food-storage* polysaccharides.

SAQ 15 No. Acid solutions (low pH) tend to disrupt the weak bonding that holds together higher-order structure. Without higher-order structure there can be no specific recognition site for adrenalin. (This is an example of the general theme: higher-order structure → specific recognition site → biological activity.)

SAQ 16 (i). Maximum activity would be seen at 50 °C and pH 7.5 even though the pH is slightly above the optimum, because at 70 °C the enzyme would be denatured. (See Section 11.2).

SAQ 17 Compound (a) is a competitive inhibitor. It resembles the substrate glucose in all respects except the orientation of one of its OH groups and can therefore still bind to the enzyme active site. (It is, in fact, galactose; Figure 5 compares the structures of galactose and glucose.)

Compound (b) is an allosteric inhibitor. It has quite a different shape from glucose, and could only be accommodated on the enzyme surface in quite a different binding site (see Section 11.5). It is, in fact, adenine (see Figure 6).

SAQ 18 (i) B. Reaction rate is proportional to enzyme concentration.

(ii) A. Boiled pepsin is denatured and therefore catalytically inactive (Sections 11.2 and 11.6).

(iii) C. All enzyme inhibitors lower the observed rate of enzyme-catalysed reactions.

(iv) C. The pseudo-peptide will sufficiently resemble the true substrate to act as a competitive inhibitor (Section 11.5).

(v) C. The pH range over which an enzyme is active is strictly defined within narrow limits. The optimum for pepsin is around pH 1–2 (see Section 11.2, and 11.7, Part IV).

SAQ 19 (a) Pepsin would clarify a meat suspension, because meat is mainly protein, and the enzyme is a broad-specificity protease, that is, any protein will act as its substrate (Section 11.3 and 11.4). (b) Pepsin (a protease) would not clarify an olive-oil suspension, as this is fat, not protein (Section 11.3).

SAQ 20 A, B, D and E. *Solution A* is needed because this contains the enzyme. *Solution B* is needed because this is the substrate. *Solution D* (buffer) is needed to maintain a constant pH—preferably near that which is optimum for enzyme X. *Solution E* (dye Y) is needed both to remove H atoms liberated during reaction and to provide a means of following the course of reaction (by measuring the absorbance changes at 340 nm).

SAQ 21 *Control tube*: 1 cm^3 A (blood) + 1 cm^3 B (compound S) + 1 cm^3 E (dye Y) + 1 cm^3 D (buffer) + 1 cm^3 H (water).

Lead-containing tube: Same as the control, but with 1 cm^3 of a lead solution (J) replacing the 1 cm^3 of water (H).

(The absolute volumes of the various solutions are not important. You may have suggested different volumes; this is permissible so long as you are sure that the *concentration* of all components is not changed by adding lead. Hence the need for 1 cm^3 of water in the control, and for this to be replaced *by exactly 1 cm^3* of lead solution in the experimental tube.)

SAQ 22 F, G or I. If absorbance remains constant, this suggests enzyme X is inhibited (by competitive or allosteric inhibition—it is impossible to distinguish between them from the data given here)—or by denaturation in acid (compare effect of HCl on egg-white protein in Section 11.7).

Answer to question in the first Audio-vision sequence

The cells in Filmstrip 23.2, Frame 8, are narrow in relation to their length and have such thick cell walls that the cell is nearly solid. The function of cells of this type, which are called fibres, is to support and strengthen the plant stem.

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